MYCOLOGIA

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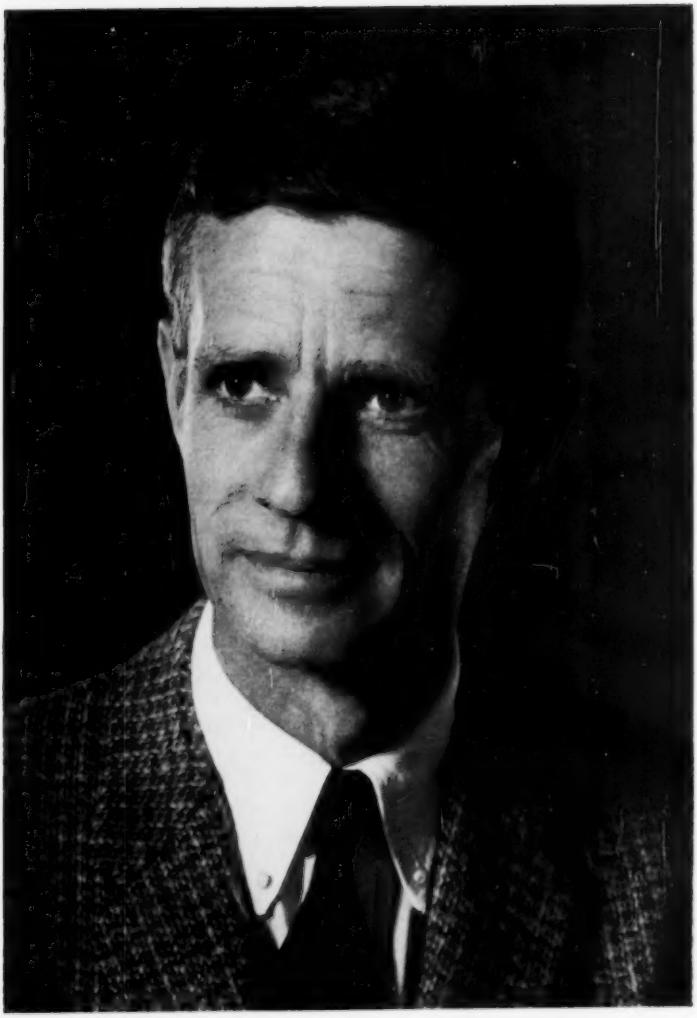

RALPH EMERSON

PRESIDENT, MYCOLOGICAL SOCIETY OF AMERICA, 1956

MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. L SEPTEMBER–OCTOBER, 1958 NO. 5

MYCOLOGICAL ORGANIZATION[1]

RALPH EMERSON[2]

(WITH 70 FIGURES)

On the occasion of our 25th anniversary, this paper is dedicated to William H. Weston, Jr., first President of the Mycological Society of America and great teacher who made the fungi live for so many of us.

INTRODUCTION

In an age when organization is revered for its own sake as never before, I hesitate to add this pebble to the pile. Nevertheless, I propose to consider here two types of organization that are of particular concern to all of us. My first point relates to botany as a whole and its bearing upon mycological organization. It has always seemed to me that botany is the study of plants from any and all points of view. It includes genetics, physiology, and biochemistry just as surely as it covers taxonomy, morphology or anatomy. None of these disciplines can stand alone and only by a synthesis of all of them will we ever reach the goals that we seek. Yet, with increasing frequency, botany is ever more narrowly

[1] Presidential address, presented at the Storrs meeting of the Mycological Society of America, August 27, 1956.

[2] During the past several years a number of graduate students have helped most ably and generously with the arduous maintenance, organization, and preparation of the cultures that are the basis of this paper. To Dorothy Ellis Sistrom, Jean McEown Crasemann, Howard Whisler, and Melvin S. Fuller I extend my warmest thanks. For the time and care they gave to the photography and final preparation of many of the figures I also wish to express deep gratitude to Howard Whisler, Melvin S. Fuller, and Kenneth Greider.

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conceived. For, if a man is interested in ion uptake by roots, he is a physiologist, not a botanist. If another investigator concerns himself with leaf differentiation, he is a morphogeneticist, not a botanist. If a third should become quite expert in hybridity, he is a geneticist, not a botanist.

Now, just as botany, a major branch of modern science, is weakened if it loses these specialists, so mycology loses strength and vigor when it fails to welcome and encourage physiologists, biochemists, allergists, industrialists, microbiologists—indeed any and all who have an interest in and do work with fungi. Our Society needs all these people; they bring it variety and new ideas, and with these qualities come greater strength and sounder productivity. Some years ago we saw the establishment of a new society of applied microbiology. This was probably inevitable and natural, but it drew off the support of many so-called applied mycologists. At the present time the fungi are playing an increasingly important role in many aspects of physiology, medicine, genetics, biochemistry, pharmacy, and other areas of experimental and applied biology. Surely we agree that we must have these fields well represented by our membership and in the pages of our journal: we must embrace the specialists in every field, both the old and the new, the obscure as well as the currently popular.

For this reason, and because science is evolving so rapidly, we should be more than ever alert to consider whether our present organization is designed to attract the full range of the best that is being done in mycology today. Frankly, some of us believe it is not and that certain fundamental operations of our Society and our journal require greater flexibility. For example, were we to modify the ruling that nonmembers cannot publish in *Mycologia*, we would be in a position to encourage new membership and wider coverage by occasionally accepting or even soliciting distinguished contributions outside our membership. Or, in view of the widening vistas of mycology, adoption of a system of specialist-reviewers like that used by other distinguished journals might aid in the selection of manuscripts and improve the quality of our publications. Annual page limits for *Mycologia* might be less rigid and we might even consider having more than six issues yearly. Indeed it is evident that these and other serious problems revolve around *Mycologia*. In this connection it is essential to understand that we have a contractual agreement with the New York Botanical Garden regarding publication of *Mycologia*, so that our control over our official organ is limited, financially and editorially. The wisdom of this arrangement has been questioned in recent years, and it is my personal belief that we should work

toward complete independence and ownership of our journal. Only in that way, I feel, can we have the flexibility of organization that will allow us to keep abreast of mycology in all its varied aspects.

And here I come to my second main point, for, in the years ahead, whatever we may do to its mechanical organization, our Society will have breadth and vigor and foresight only to the extent that we can develop these characteristics in the students who work with us. Thus it is essential to launch each student upon his career with the broadest appreciation of fungi as dynamic, functioning organisms possessing all the mysterious and fascinating features of living things. Obviously the basis for such an appreciation can come only from study of living fungi. So, when I was faced 10 years ago with the problem of presenting an advanced course on the Phycomycetes and Ascomycetes, I felt that the two weekly laboratory periods should have the largest possible proportion of living material, actively performing and representing as many key stages of growth and development as could be mustered.

Unquestionably the aquatic Phycomycetes offer some of the most exciting possibilities for fundamental laboratory study, and yet I believe I am correct in concluding that the treatment of these forms through undergraduate and graduate programs in botany is rarely adequate and sometimes goes little beyond *Saprolegnia* and *Pythium*. Why should this be so? There *are* those among us who by their native skill and many years of experience tracking the wily water mold are able to produce and demonstrate for a class an extraordinary variety of such fungi conjured each week from pools and ponds in the neighborhood or resurrected in active state from ancient jars and dried up culture dishes. But these talents are rare indeed, and how are the rest of us to organize our aquatic mycology in a reasonably pedestrian manner that can be depended upon to work with fair regularity from year to year?

The answer, I am convinced, lies in two forms of organization: first in the use of pure cultures and second in the application and extension of the principles worked out and enunciated so clearly by the great German physiologist Georg Klebs. Klebs (**1896**, **1898**, **1899**) showed many years ago that the behavior of a specific fungus is dependent upon the environment, and any particular response can usually be induced again and again at will by establishing the correct sequence of environmental stimuli. In short, it is my purpose to describe here the mycological organization we have worked out for routine demonstration of growth, differentiation, and development of some 18 genera of aquatic Phycomycetes representing 6 different orders in the monoflagellate and biflagellate series. For each genus there will be an expert somewhere

who knows far more than we. I have spent 25 years learning to know *Allomyces* with such intimacy, and I realize how little I know about other genera by comparison. If experts on these genera find our methods cumbersome and unnecessarily complicated, we will welcome their criticisms and eagerly incorporate their suggestions.

The following sections of my paper are largely concerned with the details of methods and procedures. They make dull reading for the most part except for him who is faced, as we were, with the urgency of actually preparing the cultures. To him we commend these pages and urge him to follow the directions closely for each, so far as we know, is essential. To lighten the tone, add stimulus, and provide exact documentation of the results of our organization, an extensive series of photomicrographs has been prepared. They reveal the variety and beauty of these fascinating microorganisms and in a number of instances sequences of developmental action have been caught. Yet at best they are only a shadow of what such material can reveal to the student who bends his own eye to the microscope.

MEDIA AND GENERAL METHODS

Culture flasks and dishes. While the particular glassware we have employed will not necessarily need to be exactly duplicated, conditions of aeration, nutrition, "staling," and so forth are so much affected by the culture containers used and are in turn of such critical importance in controlling growth and reproduction that precise description of the glassware will be helpful. All items are Pyrex and have the following dimensions: test tubes (tt) 150 × 20 mm; standard petri dishes (PD) 100 × 15 mm; small petri dishes (pd) 60 × 15 mm; crystallizing dishes (CD) 90 × 50 mm with a standard petri dish lid as a cover; Erlenmeyer flasks (ef) 125 ml; side-arm flasks are florence flasks (ff) 125 ml, with a side arm to carry alkali as developed and described by Cantino (**1949**).

Water. As all students of the water molds know, this is one of the most important items to be considered, and unfortunately it is still surrounded by a certain amount of mystery. Plain distilled water provides an unnatural and generally toxic environment. Some investigators (e.g., Raper, **1936**) have used charcoal-treated water, others have tried soil extracts (Goldie-Smith, **1956**), and in many laboratories autoclaved tap water has proved to be highly satisfactory (Emerson, **1941**, p. 86). A scheme we have been using with quite uniform success for many years at Berkeley involves mixing one part filtered pond water with two parts glass-distilled water, and autoclaving. This is an ill-defined mixture

that must vary significantly from pond to pond, season to season, and year to year, but even the physiologists in our laboratory have become convinced that it provides an excellent environment for water molds. Wherever water cultures are referred to here, therfore, the mixture of $\frac{1}{3}$ pond—$\frac{2}{3}$ distilled is to be used, and we routinely employ this same water in preparing all media. When I go to a new laboratory I use the normal discharge and fusion of gametes of *Allomyces* as a standard test of the water. I regret that we are still in the dark ages on the matter of water but I understand that many protozoologists and algologists are keeping us company.

Dilute salt solution (DS). In an effort to avoid the water problem, Machlis (**1953**, p. 190) devised a very dilute salt solution of the following composition: 0.001M KH_2PO_4, 0.0001M $MgCl_2$, 0.00002M $CaCl_2$, and KOH to pH 7.0. Although water is called for in most of our preparations, a few of the fungi respond more vigorously and uniformly to DS.

Baits for water cultures. The provision of a suitable substratum or "bait" for growth of fungi in water cultures is another item of critical importance. There is a nearly limitless array of possible materials to use but some are very much more satisfactory than others. A good bait provides a small, concentrated food source that is relatively insoluble, and since the total amount of dissolved nutrient in the water will often markedly influence the behavior of the fungus, care must be taken to maintain a proper balance between amount of bait and volume of water. The amounts indicated below are satisfactory for one crystallizing dish containing approximately 150 ml of water.

Hemp seeds, 2 per CD: The seeds, or more correctly the achenes, of *Cannabis sativa* L. constitute one of the best and most widely used natural substrata for growth of the common water molds. Unfortunately *C. sativa* is the source of the drug marijuana so that viable seeds must be obtained through the state or federal narcotics authorities. It is our experience that the non-viable, treated seeds supplied by pet shops are genrally unsatisfactory for water cultures.

Just prior to use the hemp seeds are boiled for 10 to 20 minutes to sterilize them and rupture the ovary and seed coats. Because heating softens the contents of the seed and renders them more soluble, the boiling must be carefully timed and the seeds must not be allowed to remain in the hot water for any appreciable time thereafter. To insure the rupture of the ovary coat without excessive cooking, the seeds are usually pinched gently after boiling to reveal the white hypocotyl within.

Grass blades, two pieces per CD: The blades of various common

grasses also provide a highly satisfactory substratum for many water fungi, certain of which do poorly or will not grow at all on hemp seeds. Young, tender blades are selected, cut into approximately one inch lengths, and boiled for 10 minutes. We have found that *Agrostis alba* L.[3] and *A. semi-verticillata* (Forsk.) C. Christ.[3] serve well for this purpose, and Dr. John Couch (personal communication) has recommended species of *Paspalum*.

Pollen grains, a small dusting on the surface of the water: Various Chytridiales and other delicate, less common aquatic fungi were originally described growing on pollen, and this substratum provides excellent growths for class study. No doubt many types of pollen would be entirely satisfactory. Clean pollen of coniferous trees can be obtained readily in ample quantities, and, after gaseous sterilization with propylene oxide, as described by Hansen & Snyder (**1947**), can be stored and used as needed over a period of some years.

Cellophane, one or two one-inch squares per CD: Following the reports by Haskins (**1939**) and others of the use of cellophane as a substratum for cellulose-decomposing Chytridiales, this material has been used increasingly because of its particularly favorable properties. It is essentially insoluble in water, so that the problem of nutrients in solution need not be a concern, and its transparency permits microscopic observation of all parts of the fungus thalli (cf. FIG. H, 5). We use wettable cellophane sheet (#300 PT, obtained from the Zellerbach Paper Company) cut in one-inch squares and sterilized by boiling in water for ten minutes. As a source of nitrogen and other essential nutrients a small piece of boiled grass blade is also provided.

Media. Whenever microorganisms of many different kinds are being isolated and kept in a collection, there is a tendency to build up requirements for a large number of special media. We have made an effort to test our new isolates systematically on certain standard media and have found that a limited number of such media meet nearly all of our needs. All solid media contain 2% plain Difco agar unless otherwise indicated, and liquid media without agar are designated as broth. Broth cultures are set up either in 125 ml Erlenmeyer flasks (ef) with 50 ml of medium per flask or in 125 ml, side-arm florence flasks (ff) with 75 ml of medium per flask.

Yeast-starch (YpSs): Powdered yeast extract (Difco), 4 g; soluble starch, 15 g; K_2HPO_4, 1 g; and $MgSO_4 \cdot 7H_2O$, 0.5 g per liter of water. This medium, originally developed for *Allomyces* (cf. Emerson, **1941**,

[3] Mr. Beecher Crampton, of the Department of Agronomy, University of California at Davis, very kindly made these determinations.

p. 87), has proved to be particularly suitable for many other fungi. A commercial preparation has recently been made available by Difco.

Yeast-glucose (YpG): Just as above but with 20 g of glucose in place of the starch.

Yeast-glucose (GY5): Powdered yeast extract (Difco), 1 g; glucose, 3 g; KH_2PO_4, 1.4 g; Na_2HPO_4, 0.6 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; and 5 ml of 0.04% aqueous bromcresol purple per liter of water. This medium is generally used in side-arm flasks (ff) as described by Cantino (**1949**) to grow organisms that produce large amounts of acid during growth and carbohydrate metabolism. Nine ml of $\frac{1}{4}$ molar NaOH are placed in the side arm and provide sufficient alkali to neutralize the cultures and hold them between pH 5.5 and 6.5. In practice, a few drops of alkali are tilted into the medium whenever the bromcresol purple begins to turn yellow.

Tryptone-glucose (TG): Powdered tryptone (Difco), 10 g; and glucose, 10 g per liter of water.

Peptone-glucose (PG): Powdered peptone (Difco), 10 g; and glucose, 10 g per liter of water.

Corn meal (BBL): Powdered corn meal agar, made up according to the specifications of the manufacturer (Baltimore Biological Laboratories), has no added glucose and is an exceptionally transparent and useful medium for growing many aquatic fungi.

General methods. Zoospores are collected and transferred by means of capillary, Pasteur pipettes and are streaked in several drops of water on agar media with a small, curved glass rod. Details will be found in the papers by Emerson and Cantino (**1948**, p. 160) and Emerson (**1950**, p. 174).

All cultures are held at room temperature (ca. 20° C) unless otherwise indicated.

Shake cultures are grown on a horizontal, rotary shaker making 170 rpm and having sufficient amplitude to cause vigorous aeration.

All photomicrographs were taken with a Jena Zeiss microscope and nearly all with phase-contrast optics. The "Orthophot" light source and camera attachment were manufactured by Silge and Kuhne of San Francisco. Eastman Kodak Panatomic X sheet film ws used and the original negatives were large enough ($2 \times 2\frac{1}{2}$ inches) for either contact printing or further enlargement as might be desired. Each of the photomicrographs depicts structures produced according to the procedures described in the present paper.

Numbering and naming of isolates. We have adopted a simple scheme of numbering in which the first number usually indicates the

Organism
Culture No.
Structures to be observed

day needed
days ahead
day start

Medium, container, temperature (other than room), and key number (multiplied by number of students gives number of cultures needed)

Special directions

Notes on effectiveness

Suggested modifications

Abbreviations
CD crystallizing dish
PD 10 cm petri dish
pd 6 cm petri dish
tt test tube
ef 125 ml erlenmeyer

Chytridium 14
47-53 5
Sporangia, opercula, zoospores 9

Grass blades, CD, 17°C, 1/4

Chytridium	14	14	14
47-53	8	6	3
Complete thalli	6	8	11

0.5% tryptone 1% agar, pd, 1/2

6 make 2 stocks and hold vertical
8 place stocks horizontal
11 43 hrs before class place several colonies of young plants in pd of water at 17°C; after 3 hrs streak zoospores

FIGS. I, 1-3.

year in which the culture was isolated or received in our laboratory and the second simply designates the particular culture. Thus, *Chytridium olla*, 47-53, was the fifty-third isolate made in 1947. Isolates of *Allomyces* are an exception to this general rule and are designated by geographical origin in accordance with a scheme used earlier (Emerson, **1941**).

The naming of isolates is a very much more difficult matter and I may as well admit my own unorthodox viewpoint on the matter. In the present rather fluid state of classification of the aquatic fungi I believe that specific epithets can often be more misleading than helpful. Where, therefore, truly monographic studies have not been made we have sometimes done no more than attempt to apply a generic designation. Even that can be a risky business: dictyuchoid cultures are apt to produce achlyoid or saprolegnioid sporangia right in class, or a new genus of chytrids may have just been erected to cover papillate forms that occasionally produce some sort of operculum. We have attempted to use the oldest acceptable names and have based our decisions largely upon Sparrow's (**1943**) treatment and other, recent, comprehensive studies.

Many of the fungi to be discussed in the following pages were collected and isolated in our own laboratory. Some, however, were provided by various kind colleagues, and in this connection I am particularly indebted to Dr. R. Y. Stanier for *Rhizophlyctis rosea* (47-51, originally from the collection at Baarn) and *Monoblepharella mexicana* (47-49, originally collected by Martha E. Springer), to Dr. John Couch for *Blastocladiella simplex* (48-8), to Dr. J. R. Raper for *Achlya ambisexualis* (50-13 and 50-15), to Dr. L. D. Leach for *Aphanomyces cochlioides* (53–17), and to Dr. J. T. Middleton for *Pythium aphanidermatum* (51-18), *P. oligandrum* (51-20), *Phytophthora drechsleri* (51-11 and 51-12), *Ph. cactorum* (51-22), and *Ph. citrophthora* (51-13).

OPERATING DIRECTIONS

The card file. During the first year I attempted to put together all the necessary information about timing, media, cultural conditions, and so forth on a master chart. It soon became apparent, however, that this scheme was too inflexible and unwieldy as a working arrangement.

FIGS. I, 1–3. Sample file cards illustrating the general form of operating directions. 1. Top card, showing the kinds and arrangement of information. 2. Middle card, showing the very simple directions for producing mature plants of *Chytridium* on grass blades in water culture. 3. Bottom card, showing the more complex directions for producing plants of *Chytridium* on tryptone agar. See text for further details.

The following year the information was abbreviated and transferred to 6×4 inch cards, which proved to be very much more helpful. Each person will have his own ideas about arranging the data, but the sample cards represented in FIGS. I, 1–3 indicate the form we have used. The most critical information is represented by the numbers in the upper right-hand corner, which show when each particular operation must be carried out. If the first day of class is arbitrarily numbered 0, the rest of the days of the semester for 15 weeks can be numbered 1 to 105 and every day will be identified. Then, if a particular culture is needed on day 14 (cf. FIG. I, *2*) and has to be prepared 5 days ahead, it must be started on day 9 (14 minus 5). All of the cards in the file are arranged in numerical sequence according to the starting day. Hence, on any given day, say day 8 for example, the cultures to be started will be shown by a glance at those few cards which have 8 as the starting day. If several manipulations are required for a given culture on different days, there will be several sets of numbers in the upper right hand corner as in FIG. I, 3. In order to prepare thalli of *Chytridium* on agar the cultures must be attended to three different times, on day 6, on day 8, and on day 11. After the stocks are made on day 6, the card is moved ahead to the 8 position; and after stocks have been placed horizontal on day 8, the card is again moved ahead, this time to the 11 position.

In recent years it has been found helpful to have a second card file showing all the media and other preparations that must be made prior to actual inoculation of the cultures. These cards too are numbered by day. The two sets of cards can be combined if desired and of course the modern system of punch cards lends itself beautifully to complete cross-indexing by name of organism, type of medium, or any other set-up that may be needed.

Since each instructor's schedule will be different, *the only day designation in the following operational directions is the number of days ahead.* The quantities of material are considered ample for a class of 12 students. The directions for keeping stock cultures of each organism are given first and are then followed by instructions for preparing the class material. The letters RS refer to resting or resistant sporangia.

CHYTRIDIALES

1. *Rozella allomycis* Foust (47-54) on *Allomyces arbuscula* Butler (Philippine Islands 1); FIGS. II, 1–4 and IX, 4.—*Stocks:* On YpSs slants; transfer every 6 weeks by blocks of agar containing infected mycelium placed in 1 ml water at base of new slant; hold tt upright ca. 7 days until

host hyphae are well established, then slant tt so that agar surface is horizontal.

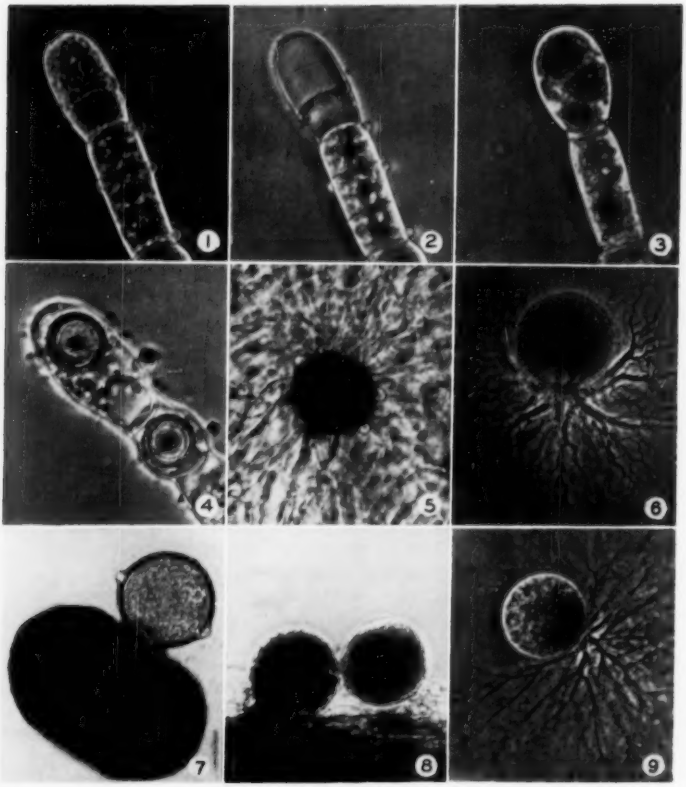
Preparation: -23 (i.e. 23 days before class), start 2 new stocks and 2 pd of *Allomyces* gametophyte (cf. item 8 below); -14, inoculate 2 water cultures, in CD plus hemp seeds, with gametangia of *Allomyces*, and after 18 hours add young *Rozella* from new stocks; (a) *-7*, for mature RS start 6 water cultures just as above but use young material from water culture as the *Rozella* inoculum; (b) -4, for zoosporangia and developing RS repeat as day *-7*. Experience has shown that heavier, more uniform, and more dependable infections occur under the more natural conditions prevailing in water cultures. Hence the rather complex schedule involving transfers from stock to water culture and then to water culture again. If desired, germinating RS of *Rozella* can also be obtained with fair dependability as follows: (c) between days -60 and -30 make several extra stock cultures at intervals of 2 weeks; early on day -1 (ca. 30 hrs. before class) place bits of stock material of various ages in water in pd.

This is a beautiful example of the parasitic, holocarpic chytrids the fundamental biology of which is so little known. Host-parasite relations can be considered, the basic morphological similarities between host and parasite are striking, and the likelihood of some sort of heterothallism is shown in the work of Sörgel (**1952**). The zoospores are extremely small (cf. FIGS. IX, 4–5) and active.

2. *Rhizophlyctis rosea* (de Bary and Woronin) Fischer (47-51); FIG. II, 5.—*Stocks:* On PG slants; transfer every 6 weeks by colonies of plants placed in 1 ml water at base of new slant; hold tt upright but roll water gently over agar surface twice daily until new colonies are well established; then slant tt so that agar surface is horizontal (cf. Crasemann, **1954**, p. 302).

Preparation: (a) -14, for demonstration of pigment production (carotene, cf. Cantino and Hyatt, **1953**) start 2 stock slants. An even more impressive display of pigmentation is revealed in TG broth cultures in ef, begun -14 and held at 25° C on a rotary shaker. (b) -2, for mature plants, start 4 water cultures in CD with 1 grass blade and 2 wettable cellophane.

The multipolar origin of rhizoids and the interbiotic nature of this common monocentric chytrid are well shown against the transparent cellophane. The conspicuous cellulose-decomposing ability of *Rhizophlyctis* is revealed and the nutritional significance of the grass blade can be discussed.



FIGS. II, 1–9. Chytridiales. 1–4. *Rozella allomycis* in hyphal tips of *Allomyces arbuscula*. 1 (× 375). Thin-walled zoosporangia shortly before zoospore release; spores in the terminal sporangium are fully cleaved. 2 (× 375). The same, about 15 minutes later; zoospores have been discharged from the two terminal sporangia. 3 (× 375). Thalli beginning to form resistant sporangia. 4 (× 563). Two mature resistant sporangia showing the spiny outer wall and large central globule. 5 (× 300). *Rhizophlyctis rosea* on cellophane in water culture; a mature thallus with three discharge papillae in focus. 6, 7. *Rhizophydium sphaerotheca*. 6 (× 187). A developing thallus on YpSs agar, revealing the much branched rhizoidal system. 7 (× 375). Mature thallus on a pollen grain of *Pinus* sp.; discharge papillae are shown. 8, 9. *Chytridium olla*. 8 (× 225). Two mature thalli on a grass blade in water culture; the refractile dome of the operculum can be seen on the thallus to the right. 9 (× 225). A young plant grown on tryptone agar to show the basal tuft of tapering rhizoids.

3. *Rhizophydium sphaerotheca* Zopf (55-1); FIGS. II, 6–7.—*Stocks:* On YpSs slants; transfer as (2) above.

Preparation: (a) -7, for plants on agar start new stocks; -5, slant new stocks; -2, put several colonies of young plants in pd of water; zoospores discharged in ca. 3 hours are then streaked on 6 pd of YpSs agar. (b) -3, for plants in water culture start 2 CD of water with pine pollen very lightly dusted on the surface.

On pollen this is a classic monocentric chytrid growing on a classic substratum. The several discharge papillae stand out clearly and zoospore formation and release can usually be seen. On agar the richly developed rhizoidal system is very evident.

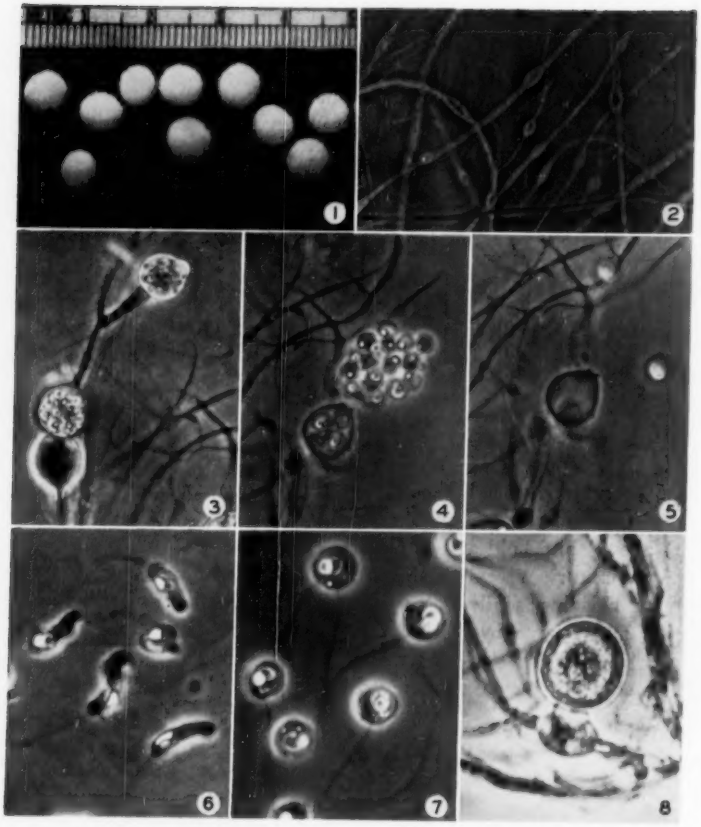
4. *Chytridium olla* Braun (47-53); FIGS. II, 8–9.—*Stocks:* On YpSs slants; transfer as (2) above.

Preparation: (a) -14, for pigment (to compare with (2a) above) start 2 new stocks. (b) -8, for plants on agar start 2 new stocks; -6, slant new stocks; -3, put several colonies of young plants in pd of water at 17° C; zoospores discharged in ca. 3 hours are then streaked on 6 pd of 0.5% tryptone plus 1.0% agar. (c) -5, for plants in water culture start 3 CD with grass blades (preferably floating) or pine pollen (as (3b) above) and hold at 17° C.

Agar cultures reveal the beautiful basal tuft of very fine, branched, tapering rhizoids; while water cultures show all stages of zoospore formation and release by dehiscence of the large, distinctive operculum. A characteristic brownish pigment accumulates in older stock cultures but its chemical nature remains to be determined.

5. *Nowakowskiella elegans* (Nowak.) Schroeter (50-1); FIGS. III, 1–8.—*Stocks:* On YpSs slants; transfer a block of rhizomycelium every 6 weeks. The so-called rhizomycelium penetrates slowly through the agar but is so fine that it is not always readily detectable with the naked eye. Assurance of satisfactory transfer can be obtained by examination with a dissecting microscope, which reveals the fine rhizoidal strands and numerous spindle-shaped swellings. No reproductive bodies are formed under these conditions, however.

Preparations: (a) -39, for RS start 4 pd of YpSs; -21, inoculate 6 TG broth cultures, each with ca. 10 little blocks (ca. 1 mm^3) cut from YpSs plates, and place on rotary shaker at 25° C; by -14, there will have been formed in each flask several large balls of rhizomycelium which are then washed through 2 changes of water, 30 min. each, and placed in water in PD at 17° C. The water should be just deep enough to cover all but the tops of the balls. There must be no more than 4 balls per dish and special precautions should be taken to avoid contamination.

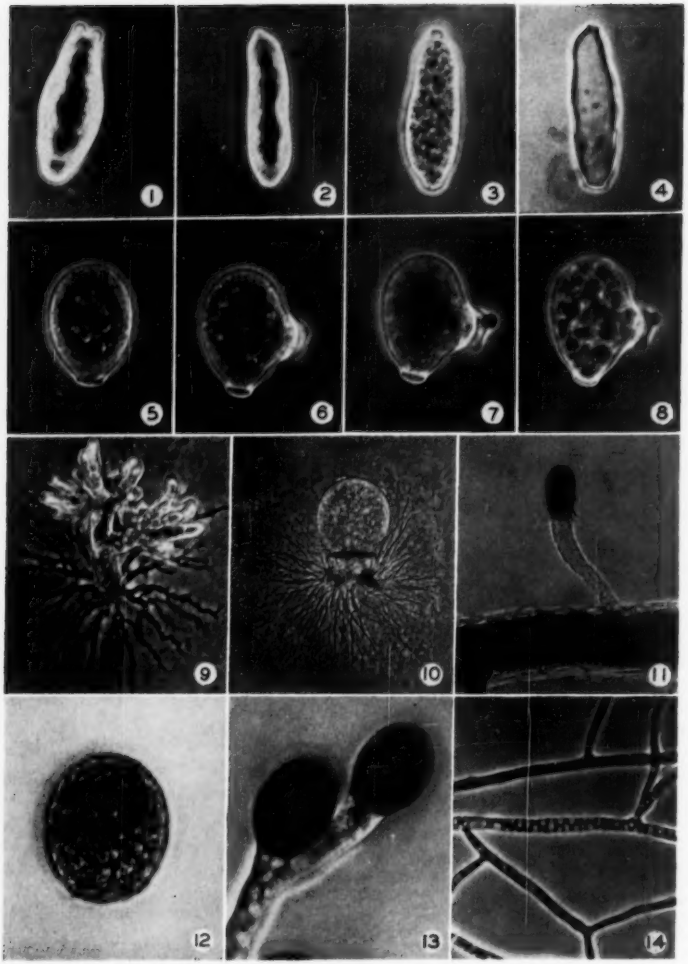


FIGS. III, 1–8. Chytridiales; *Nowakowskiella elegans*. 1. Balls of rhizomycelium formed in tryptone-glucose broth on a rotary shaker. 2 (×300). Strands of rhizomycelium with characteristic swellings; from YpSs agar. 3 (×375). Two mature sporangia showing the cleaved zoospores within; the lower sporangium rests upon a large apophysis whereas the upper sporangium is virtually non-apophysate and shows the protruding, refractile operculum. 4 (×375). A cluster of zoospores has just been released from the sporangium; the operculum has become detached and can be seen to the left of the sporangial opening. 5 (×375). The same field a few minutes later: the zoospores have gone, leaving the empty sporangium and its detached operculum. 6 (×635). Zoospores exhibiting creeping, amoeboid movements. 7 (×705). Rounded zoospores, as they usually appear just after release from the sporangium, and showing the conspicuous and highly refractile oil globule. 8 (×750). A resting spore with its thickened wall and granular protoplasm (see text).

(b) -15, for zoosporangia, from YpSs cultures started -39, again inoculate 6 TG broth cultures as above; -8, wash balls and place in water in PD just as before but now hold at room temperature. Do not disturb until ready for class.

Nowakowskiella serves as an excellent illustration of a polycentric operaculate chytrid with apophysate reproductive bodies. Several aspects of the preparations merit special comment. The bulk of rhizomycelium produced in the balls formed in liquid shake cultures is astonishing, more particularly in view of the very tenuous appearance of the growth on agar slants. Following washing and transfer to water, a delicate new growth extends out from the balls, particularly along the surface of the water, and it is here that numerous sporangia are produced at room temperature. They range all the way from fully apophysate to virtually non-apophysate; they show all stages in the formation and discharge of the unusually large and beautiful zoospores with their conspicuously refractile central body. The curved opercula can be readily observed before, during, and after spore release.

A feature of particular significance is the induction of what appear to be resting spores or resistant sporangia by subjection of cultures to a slightly reduced temperature (17° C). Under these circumstances many fewer zoosporangia are produced, there is a less extensive new growth of rhizomycelium, and in clumps and clusters about the balls are formed large numbers of smaller, globose bodies sometimes apophysate but lacking any suggestion of an operculum. By the end of 14 days these structures have formed a slightly thickened and pale yellowish wall. The cytoplasm is fairly evenly granular and does not contain the refractile bodies so characteristic of mature zoosporangia. Sparrow (**1933**; **1943**, pp. 380–381) commented upon and figured "thick-walled spherical or ellipsoidal apophysate colorless resting spores with fatty contents found associated with the sporangia" in his British collections of *N. elegans*. While he believed they might belong to this species he could not be certain, and in his key to the species (**1943**, p. 379) he states that *N. elegans* does not form resting spores. I believe our pure cultures show conclusively that resistant spores or sporangia are indeed produced directly on the rhizomycelium of *N. elegans* and that they are initiated in response to evironmental conditions, specifically, lowered temperature, that limit zoosporangium formation and enhance the production of RS.



FIGS. IV, 1–14. Blastocladiales and Monoblepharidales. 1–9. *Blastocladia ramosa*. 1–4 (× 450). Zoosporangia: 1, with two discharge papillae; 2, with a single discharge papilla; 3, with spores cleaved and nearly ready to emerge; 4, empty sporangial wall after spores have gone. 5–8 (× 563). Resistant sporangia; 5, in resting condition; 6, shortly after germination has started, showing split outer wall and protruding inner membrane on which discharge papillae are beginning to form; 7, 110 minutes later the tongue-like protrusion has reached its full size and the two papillae are well formed; 8, by 10 minutes more the zoospores have become

BLASTOCLADIALES

6. *Blastocladia ramosa* Thaxter (54-8); FIGS. IV, 1–9.—*Stocks:* On GY5 broth in ff; transfer by pipetting a few plants into new broth every 6 weeks, neutralize with alkali from the side arm twice daily during active growth, and hold at 17° C.

Preparation: (a) -90, for germinating RS start 2 stock cultures on GY5 broth as above, neutralize when necessary, and hold at 17° C; 0, 6 hours before class, pipette aliquots of plants and deciduous RS into 2 CD of water. (b) -7, for plants of all sizes and discharging zoosporangia start 2 stock cultures as above; 0, 2 hours before class, pipette aliquots of plants into 2 CD of water. (c) -1 (about 32 hours before class), for germlings on agar pipette an aliquot of plants from stocks prepared in (b) into CD of water; 2 to 3 hours later copious zoospore discharge will have occurred and spores can be streaked on half-strength GY5 with 1.5% agar in 12 pd.

Here again several points of interest are raised. Both zoosporangia and RS are highly deciduous. The strain of *B. ramosa* we are using was collected in the Sacramento Slough, California, and isolated by Howard Whisler. Previously our only isolates had come from Cambridge, Massachusetts (cf. Emerson and Cantino, **1948**; Emerson, **1950**). 54-8 has all the morphological characteristics of *B. ramosa* as described by Thaxter (**1896**) and as exhibited in our Cambridge collections. Like them it produces RS with generally very thin walls, little if any pigmentation, and very faint pitting. On the average, however, the walls are just enough thicker, show sufficiently distinct pitting on careful observation, and develop enough pale smoky pigmentation so that we can be much more certain of their resistant-sporangial nature. It is a striking fact, therefore, that no special treatment with carbon dioxide, like that

fully cleaved; discharge occurred very shortly thereafter. 9 (× 225). A young plant on agar; the basal rhizoidal system is well developed and the first sporangia are starting to form on the branches of the main thallus. 10–12. *Blastocladiella simplex*. 10 (× 75). Mature thallus on agar; a large zoosporangium has been produced and two papillae are shown. Under these conditions the thalli are so shortened as to give the appearance of a chytrid, yet the septum that separates the zoosporangium from the short vegetative part of the main thallus is clearly evident. 11 (× 75). A plant that has developed in water culture on grass blade shows the markedly more elongate thallus characteristic of these cultural conditions. The sporangium is not yet fully differentiated. 12 (× 375). Mature resistant sporangium from water culture; the coarse network of sculpturing on the outer wall is just apparent. 13 (× 375). Resistant sporangia of *Allomyces macrogynus* for comparison. 14 (× 563). *Monoblepharella mexicana* mycelium with the "foamy" vacuolate cytoplasm that is so distinctive in this genus and *Monoblepharis*.

reported previously for *B. pringsheimii* (Emerson and Cantino, **1948**; Cantino, **1949**; Emerson, **1955**) was required for the production of RS in pure cultures. These structures are formed in profusion in ordinary GY5 broth cultures of *B. ramosa*. Moreover, after a period of aging or maturation, the length of which has not been definitely established yet but is in the range of 30 to 90 days, many RS will germinate when transferred from broth culture to water. As regards the formation and germination of RS in pure culture without special treatment, *B. ramosa* is, therefore, like *Allomyces* spp. and unlike *Blastocladia pringsheimii*. Noteworthy also is the fact that, unlike any other described species of *Blastocladia*, zoosporangia with more than one discharge papilla (FIG. IV, 1) can frequently be found in *B. ramosa*. This too is a feature characteristic of *Allomyces* and *Blastocladiella*. Nevertheless in its metabolism and nutrition (Emerson and Cantino, **1948**; Crasemann, **1957**) as well as in the manner of its RS germination by a tongue-like protrusion (cf. Blackwell, **1940**) *B. ramosa* proves itself to be a true *Blastocladia*. It holds promise of revealing significant aspects of the life cycle of that still too obscure genus.

7. *Blastocladiella simplex* Matthews (48-8); FIGS. IV, 10–12.—*Stocks:* on YpSs slants; transfer as (2) above.

Preparation: (a) -77 and -56, for germinating RS prepare 2 stocks; 0 (about 7 hours before class), place RS in pd of DS. (b) -7, for plants in water culture with zoosporangia and RS start 3 CD with 1 grass blade; -4, repeat. (c) -1, for plants on agar showing rhizoidal system and zoospore release, 30 hours before class place RS (from (a) above) in pd of DS; after 6–7 hours streak zoospores in 12 pd of YpSs/2.

Because of its relationship to *Allomyces* and the extensive experimental work being done with it by Cantino and his co-workers (cf., for example, Cantino, **1956**), *Blastocladiella* is of particular interest. It would be nice if one could demonstrate isogamy and the entire life cycle of equal alternating generations as described by Harder and Sörgel (**1938**) in *B. variabilis*. However, many features of this cycle remain unclear. Our isolate, 48-8, was sent to us by Dr. Couch presumably as a *B. simplex* with the short, asexual (*Brachyallomyces*) cycle. Yet spores from RS give a small percentage of orange plants. Just as in Cantino's *B. emersonii*, however, swarmers from the orange plants do not behave as male gametes: no attraction to colorless female (?) swarmers has been observed and no positive evidence of syngamy has been obtained. The range in thallus form resulting from slight environ-

mental differences is very striking. The factor involved is probably aeration.

8. *Allomyces* (*Euallomyces*) *macrogynus* (Emerson) Emerson and Wilson (Burma 1Da); FIGS. IV, 13 and IX, 5.—*Stocks:* On YpSs slants; transfer every six weeks by small blocks of mycelium carefully cut from the lower end of the slant and placed, right side up, at the upper end of the new culture. If precautions are taken to avoid sudden temperature changes and the consequent condensation of moisture, haploid (gametophytic) and diploid (sporophytic) stocks can be kept with little difficulty. Test tubes of new media should be made up and slanted about 4–7 days ahead so that the agar surface will dry before inoculation. Large numbers of viable reproductive structures can readily be removed from agar cultures by gently scraping the surface with a flattened transfer wire.

Preparation: (a) -84, -77, and -70, for germinating RS make 2 sporophytic stocks; 0, place scrapings of RS in water in pd 1–2 hours before class. (b) -14, for mature sporophytic thalli bearing both zoosporangia (mitosporangia) and RS (meiosporangia) in water culture, start 2 new gametophytic stocks; -4, inoculate 4 pd with 1 hemp seed with small scrapings of gametangia; -3, move each seed to new water in pd. (c) -10, for gametangia, gamete release, and syngamy, start 3 haploid cultures on YpSs in pd with transfers from gametophytic stocks prepared -16; 0, 1 hour before class, put scrapings of gametangia in water in pd. (d) -1, for germling plants showing early development and polarity, put scrapings of gametangia in water in pd 27 hours before class and streak zygotes on pd of YpSs/2 22 hours before class; repeat these operations about 8 hours later.

Euallomyces is the most satisfactory organism I know of to illustrate the basic features of sexual and asexual reproduction. At all levels, from general high school biology to advanced graduate study in mycology, for classes of half a dozen or several hundred, the formation, behavior and development of the four types of reproductive organs and motile swarmers can be demonstrated with complete regularity and dependability. I have written so much about this organism in recent years, and all stages of its life history have been so fully illustrated (Emerson, **1941**, **1955**) that I shall resist the temptation to comment further here. It is noteworthy, however, that all major aspects of the life cycle of *Euallomyces* have been captured in a phase contrast motion picture made several years ago in our laboratories by Arthur T. Brice of Phase Films in Sonoma, California. Although the sound-commentary and emphasis in "Syngamy and the Alteration of Generations in *Allo-*

myces" are directed toward college classes in general biology, the detailed portrayal of growth and development can be a source of great stimulation to advanced students as well.

MONOBLEPHARIDALES

9. *Monoblepharella mexicana* Shanor (47-49); FIGS. IV, 14 and V, 1–3.—*Stocks:* Two methods have been employed; (a) in 0.5% tryptone broth in ef; transfer tufts of mycelium every 6 weeks; or (b) on BBL slants; transfer a block of mycelium every 6 weeks. The slant cultures are less likely to become contaminated and are easier to carry but their viability may not be so reliable. Growth is much more vigorous but viability is definitely less on media containing glucose. Both *Monoblepharella* and *Monoblepharis* have special nutritional requirements and to my knowledge no chemically defined medium has yet been worked out that will support consistently good growth.

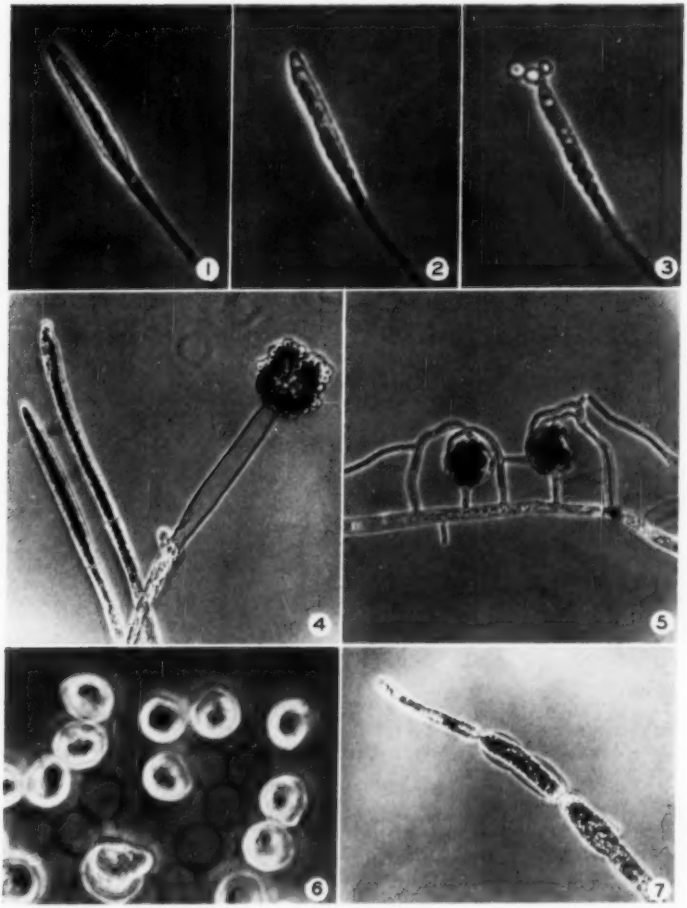
Preparation: -5, for zoosporangia and spore release start 6 stock broth cultures; -1, 28–30 hours before class, wash the delicate mycelium through two changes of water, 10 minutes each, and then place in 6 CD of water. The fine hyphae are difficult to handle with ordinary transfer needles. Glass rods are more satisfactory, and washing can also be facilitated by holding material in small baskets made of 100 mesh, 0.0045 gauge, monel wire cloth, like those used by Machlis (**1953**).

The characteristically vacuolate hyphae and delicate spindle-shaped sporangia of the Monoblepharidales can be well shown in this material, but it has been a disappointment to us that we have not yet succeeded in obtaining the spectacular sexual stage of any of these forms in pure culture.

SAPROLEGNIALES

10. *Saprolegnia* sp. (51-1); FIG. V, 7.—*Stocks:* On BBL slants; transfer every six weeks by blocks of mycelium cut from the lower end of the slant and placed at the upper end of the new culture.

Preparation: (a) -10, for proliferating zoosporangia and release of primary zoospores start 2 stock water cultures from agar stock by placing agar block with mycelium in CD of water with 2 hemp seeds (one seed should remain in contact with the inoculum); -3, using tufts of mycelium from stock water cultures as inoculum, start 6 CD with hemp. Almost any *Saprolegnia* isolated from soil or muddy waters anywhere will show the characteristic sporangia and zoospores when treated in this general manner. Many isolates will also form gametangia in



FIGS. V, 1–7. Monoblepharidales and Saprolegniales. 1–3 (× ca. 400). *Monoblepharella mexicana* zoosporangium: 1, with single terminal exit papilla; 2, with spores cleaved and about to emerge; 3, first spores emerging. 4–6. *Achyla* sp. 48-2. 4 (× 115). Three typically clavate zoosporangia; the one on the right has discharged and the primary spores are encysted at the apex. 5 (× 115). Two oogonia with numerous antheridia and developing oospores. The monoclinous condition is clearly demonstrated in this isolate. 6 (× 750). Encysted primary zoospores releasing secondary zoospores. 7 (× 120). *Saprolegnia* sp. 51-1. Zoosporangium that has proliferated several times to produce the appearance so characteristic of this genus.

slightly older cultures. Our demonstration of sexual structures in the Saprolegniaceae is usually made with other genera (see below). (b) An alternative method, requiring more precise timing but yielding great numbers of spores, is as follows: -2, inoculate 2 ef of YpG broth with transfer from agar stock and place on shaker at 25° C; day 0, 5 hours before class, wash the mycelia through three changes of water, 10 minutes each, and place in 4 CD of water. (c) -10, for gemmae inoculate 2 ef of YpG broth and place on shaker at 25° C; -7, divide the mycelium into small tufts, wash through 3 changes of 1% NaCl, 30 minutes each, and place in 2 CD of 1% NaCl. This method, first worked out by Klebs (**1899**), will probably effect the formation of gemmae by many of the common Saprolegniaceae.

11. *Achlya* sp. (48-2); FIGS. V, 4–6.—*Stocks:* On BBL slants; transfer as (10) above.

Preparation: (a) For zoosporangia, zoospores, just as (10a) above. (b) As an alternative, the method described in (10b) above is also very effective. (c) -14, for oogonia, oospores and monoclinous antheridia start 1 water stock as described in (10a) above; -7, with inoculum from water stock start 2 CD with hemp. All stages in the development of the characteristic gametangia of the Saprolegniales will occur in such cultures.

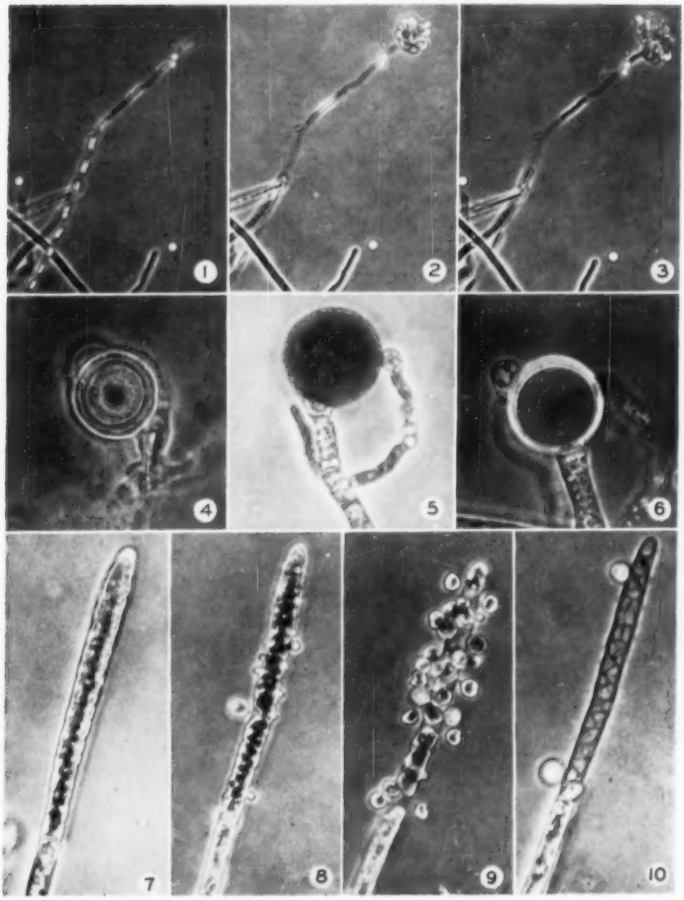
12. *Achlya* sp. (47-5).—*Stocks:* On BBL slants; transfer as (10) above.

Preparation: For oogonia, oospores, and diclinous antheridia, exactly as (11c) above. In my experience *Achlya* is the commonest of all water molds and turns up over and over again in soil-water cultures. Sexual isolates regularly produce gametangia when prepared as described here.

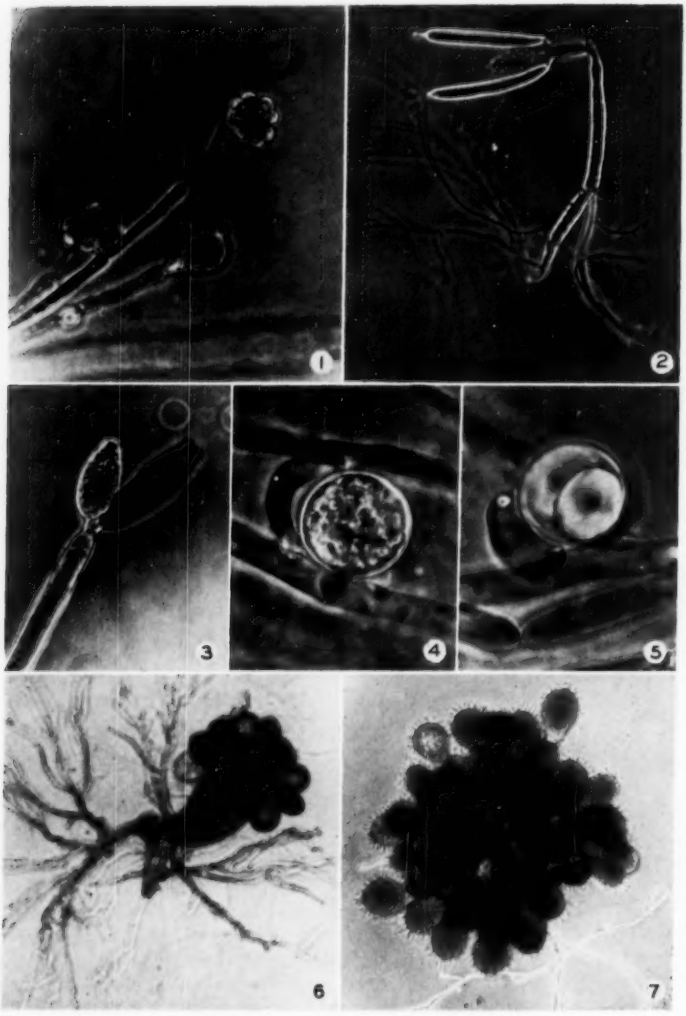
13. *Achlya ambisexualis* Raper (50-13 ♂ × 50-15 ♀).—*Stocks:* On BBL slants; transfer as (10) above.

Preparation: -14, to demonstrate heterothallism inoculate one pd of BBL with the ♂ and one with the ♀; -7, cut blocks (ca. 5 mm²) from the BBL plates and inoculate 4 PD of Raper's medium (soluble starch, 3 g; inositol, 1 g; peptone, 1 g; agar, 20 g; water, 1 liter) each with two blocks placed ca. 3 cm apart, 2 plates ♀ × ♂, 1 plate ♂ × ♂, and 1 plate ♀ × ♀; -5, inoculate 4 more PD of Raper's medium just as above. These cultures provide a simple demonstration and basis for discussion of John Raper's (**1951**) classic observations on the hormone-controlled sequence of events in the sexual reproduction of *Achlya*.

14. *Dictyuchus* sp., an asexual (heterothallic ?) strain (47-6); FIGS. VI, 7–10.—*Stocks:* on BBL slants; transfer as (10) above.



FIGS. VI, 1–10. Saprolegniales. 1–3 (×150). *Aphanomyces* sp. 53-15b. Three stages (about one minute apart) in the release of primary zoospores and their encystment at the orifice of the zoosporangium as in *Achlya*. 4 (×600). *Aphanomyces cochlioides* oogonium with antheridium and single nearly mature oospore which shows a thickened wall and centrally located reserve globule. 5, 6 (×375). *Dictyuchus* sp. 48-6. Oogonia and attached antheridia; 5 presents an early stage at about the time of fertilization, while 6 shows a maturing oospore. 7–10 (×225). *Dictyuchus* sp. 47-6. A zoosporangium shown at about three-minute intervals during the release of secondary zoospores. The network of empty cyst walls shows well in the last figure, and many of the small, individual exit pores can be seen.



FIGS. VII, 1–7. Leptomitales. The characteristic hyphal constrictions are evident. 1, 4, 5. *Apodachlya* sp. 47-17. 1 (× 233). Zoosporangia, one with fully developed papilla and about to discharge, the other two with discharged primary zoospores encysted at the apex. 4 (× 750). Antheridium and oogonium containing immature oospore. 5 (× 750). Antheridium and oogonium containing mature

Preparation: (a) -13, for dictyuchoid sporangia in all stages of development and discharge prepare a water stock as in (10a); -6, prepare 6 CD with hemp as in (10a). (b) Alternatively, broth cultures may be used here too and a possible schedule is as follows: -4, inoculate 6 ef of YpG broth with transfer from agar stock and place on shaker at 25° C; 0, 8 to 10 hours before class, divide the mycelia into small tufts, wash through three changes of water, 10 minutes each, and place in 6 CD of water.

15. *Dictyuchus* sp., a sexual, homothallic strain (48-6); FIGS. VI, 5–6.—*Stocks:* On BBL slants; transfer as (10) above.

Preparation: -15, for monosporous oogonia, oospores, and monoclinous antheridia, prepare a water stock as in (10a); -8, prepare 3 CD with hemp as in (10a). Although this culture has typical dictyuchoid gametangia and sporangia in 8-day cultures, at 3–5 days many sporangia are discharging in the saprolegnioid manner. Such material can be used effectively to emphasize the puzzling intergeneric intergradations stressed by Pieters (**1915**) and Salvin (**1942**) but so generally ignored by taxonomists of the Saprolegniaceae.

16. *Aphanomyces* sp. (53-15b); FIGS. VI, 1–3.—*Stocks:* On BBL slants; transfer as (10) above.

Preparation: -2, for zoospore discharge inoculate 3 ef of YpG broth with transfer from agar stock and place on shaker at 25° C; 0, 5 to 7 hours before class, divide mycelium into small tufts, wash thoroughly through three changes of water, 10 minutes each, and place in 3 CD of water. The thoroughness and timing of the washing are critical, and, in the last analysis, each instructor will probably have to work out the details of the exact schedule he uses. However, like the formation and release of zoospores in *Pythium*, zoospore discharge in *Aphanomyces* is a most exciting event and one well worth the special effort needed it produce it.

17. *Aphanomyces cochlioides* Drechsler (53-17); FIG. VI, 4.—*Stocks:* On BBL slants; transfer as (10) above.

Preparation: -21, for mature gametangia and oospores start 6 pd of BBL. Like so many other members of the biflagellate series, and as

oospore with conspicuous eccentric oil globule. *2, 3. Sapromyces elongatus. 2* (× 150). Germling on agar showing the basal rhizoids, early hyphal development, and an initial small sporangium between the first dichotomous branches. *3* (× 233). A pair of zoosporangia, one young and starting to form a papilla, the other empty. *6, 7* (× 115). *Mindeniella spinospora* from agar cultures. *6.* Younger plant with heavy basal rhizoids and developing sporangia. *7.* Older plant with the distinctive spiny sporangia.

Klebs (**1899**) showed with *Saprolegnia,* mycelia of *Aphanomyces* often form a copious crop of gametangia in agar culture. So far as I know the exact nature of the stimulus responsible, whether reduced oxygen, raised CO_2, or whatever, is not known.

LEPTOMITALES

18. *Apodachlya* sp. (47-17); FIGS. VII, 1, 4, 5.—*Stocks:* On BBL slants; transfer as (10) above.

Preparation: (a) -7, for zoosporangia and zoospores inoculate 2 ef of 0.5% tryptone broth; -1, 18–26 hours before class, wash the mycelium through three changes of water, 10 minutes each, and place in 4 CD of water at 17° C. Here again the exact timing will depend somewhat on other conditions and will have to be worked out on the spot. It is interesting to note that, whereas the mode of spore discharge has been used as a taxonomic criterion of specific value in *Apodachlya,* this strain has mostly achlyoid spore discharge at 17° C and saprolegnioid discharge in identical cultures held at 20°–22° C. (b) -20, for gametangia and oospores inoculate 2 pd of BBL; -10, use vigorous young hyphae from BBL pd to start 6 pd of GY5/2 agar. Despite the small size of the gametangia, all stages in gametogenesis and oospore maturation can be studied in these cultures. The transition from the scattered oil reserves of the young oospore to the single, large, eccentric oil drop of the mature ones is particularly well shown.

19. *Mindeniella spinospora* Kanouse (54-11); FIGS. VII, 6–7.—*Stocks:* In GY5 broth in ff; transfer every 3 months by teasing apart tufts and transplanting to new medium; hold at 17° C and neutralize as necessary.

Preparation: -14, for thalli and sporangia inoculate 3 ff of GY5 broth, hold at 17° C and neutralize as required; -4, remove the plants from one broth culture, wash through 3 changes of water, 10 minutes each, and then leave in CD of water for several hours until zoospores are produced, at which time streak 6 pd of BBL; -3, repeat as on -4. The spiny sporangia of this little organism are particularly interesting. As I reported at a meeting of the Mycological Society of America several years ago, the presence or absence of spines and the shape of the thallus as well are dependent upon environmental factors.

20. *Sapromyces elongatus* (Cornu) Coker (54-1); FIGS. VI, 2–3 and IX, 6.—*Stocks:* In GY5 broth in ff; transfer tufts of mycelium every 6 weeks, hold at 17° C, and neutralize as necessary.

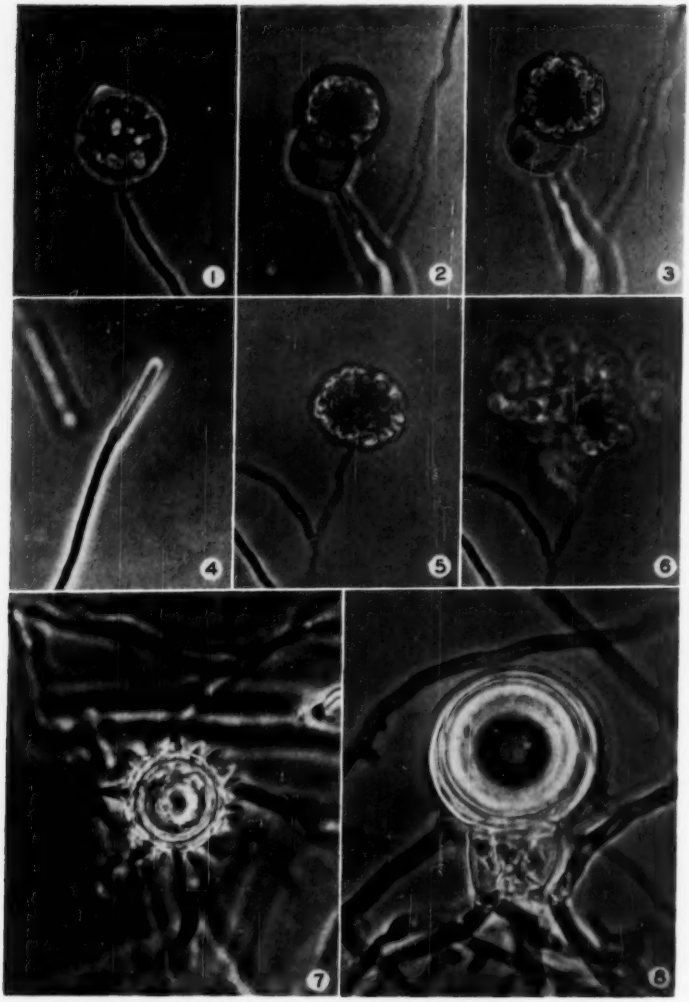
Preparation: (a) -6, for mature thalli, zoosporangia, and zoospores inoculate 2 ff of GY5 broth and neutralize thereafter as required; -1, ca.

18 hours before class, divide mycelium into small tufts, wash in water and place in 2 CD of water at 17° C. (b) -2, for germling plants showing early development and polarity rinse a little of the growing mycelium from the broth cultures (a, above) and place in CD of water for zoospore production; -1, ca. 27 hours before class, streak about 100 zoospores per plate on 6 pd of GY5/2 agar. The large, vigorous thalli and asexual reproductive structures of *Sapromyces* make a worthwhile addition to the available cultured material in this generally little known family, the Rhipidiaceae. We cannot provide sexual stages because we still do not know what conditions will induce the formation of gametangia in pure cultures of *Sapromyces* or *Rhipidium*.

AQUATIC PERONOSPORALES

21. *Pythium* spp.—*Stocks:* All strains are carried on BBL slants and transferred every six weeks as (10).

Preparation: (a) *P.* sp. (55-2); FIGS. VIII, 1–3. -9, for sphaerosporangia and zoospores start a stock water culture in CD with grass blades (one blade should remain in contact with the inoculum); -3, cut stock grass blades into pieces to inoculate 6 new CD of water and grass blades; 0, about 4 hours before class, replace the water. (b) *P.* sp. (47-11); FIGS. VIII, 4–6. -11, for nematosporangia and zoospores start stock water culture as above; -4, start 6 new water cultures as above; 0, 3 to 5 hours before class, change water three times at ½ hour intervals and then let the grass and mycelia stand in water ca. 1 cm deep. One of mycology's real thrills is to watch a *Pythium* sporangium produce spores. First is the sudden outward flow of protoplasm and formation of the delicate vesicle; after a short pause the dim cleavage outlines of the big spores can be made out; they stir and rock gently; then the motion becomes more and more urgent until they are bumping about like "cats in a sack"; and finally they burst out and scatter in every direction. The entire process may take only 5 or 10 minutes and is sometimes even more rapid. Apparently the first steps, several hours before actual discharge occurs, are set in motion by the provision of fresh water, and not infrequently several serial washes are critical as with 47-11. (c) *P. aphanidermatum* (Edson) Fitzpatrick (51-18). -11, for inflated filamentous sporangia, aplerotic oospores, and smooth oogonia with paragynous antheridia start pd of BBL; -5, start 6 PD with water and one grass blade by using a 5 mm^2 block of inoculum (from BBL plate) and laying one end of the grass blade directly on it. Fine zoospore formation can usually be induced in this material by washing



FIGS. VIII, 1–8. Peronosporales. 1–3 (× 375). *Pythium* sp. 55-2. 1. Papillate sphaerosporangium about to discharge. 2. Empty sporangium with attached vesicle in which the outlines of the spores are just becoming evident. 3. The same about two minutes later; the clearly defined spores are beginning to move about vigorously. 4–6 (× 375). *Pythium* sp. 47-11. 4. Apex of a mature nematosporangium. 5. Zoospores at a stage corresponding to FIG. 3. 6. The same about two

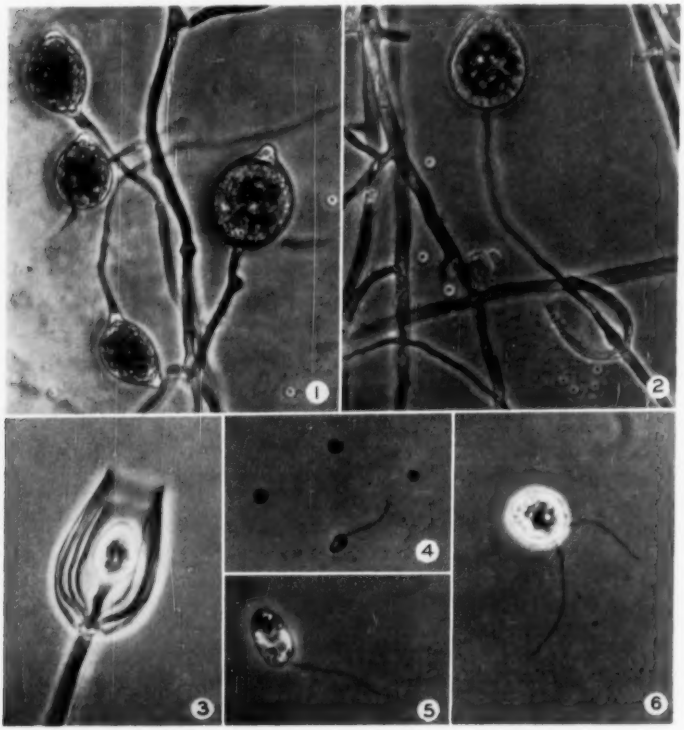
the mycelia just as in (b) but about 6 to 8 hours before class. (d) *P. oligandrum* Drechsler (51-20); FIG. VIII, 7. -13, for spiny oogonia with paragynous or apandrous antheridial condition start pd of BBL; -7, start 6 PD with DS solution and two hemp seeds by using a 5 mm² block of inoculum from the BBL plate and placing one hemp seed directly on the block. (e) *P.* sp. (47-4). -27, for smooth oogonia with plerotic, centric oospores and paragynous antheridia start new stock on BBL; -17, using agar blocks cut from the new stock, inoculate 3 CD of DS solution with 2 hemp seeds (one seed should remain in contact with the inoculum).

22. *Phytophthora* spp.—*Stocks:* All strains are carried on BBL slants and transferred every six weeks as (10).

Preparation: (a) *Ph.* sp. (47-1); FIGS. IX, 2–3. -13, for proliferating zoosporangia and direct discharge of zoospores start pd of BBL; -6, start 12 PD with DS solution and two hemp seeds by using a 5 mm² block of inoculum, from the BBL plate, and placing one hemp seed directly on the block; -2, transfer hemp seed with attached mycelia from the 12 PD to 12 CD of water. Typically aquatic, saprophytic strains of *Phytophthora* like this, with copiously formed and conspicuously proliferating sporangia, are of common occurrence in soil-water cultures. They show the characteristic, direct discharge of spores and serve to emphasize the essentially aquatic nature of most species in this economically important genus. All of our own isolates have been entirely asexual, probably as a result of the situation discussed in recent years by Waterhouse (**1953**), Stamps (**1953**), and others who have found that gametangia may sometimes be induced to develop only when two strains are brought together. The basis for this reaction is still not fully understood and the peculiar features are further emphasized by the "matings" described below. (b) *Ph. drechsleri* Tucker (51-11 "♂," and 51-12 "♀"); FIG. VIII, 8. -13, for oogonia and amphigynous antheridia start pd of BBL of each strain; -9, cut 5 mm² blocks from the BBL plates, place 1 block of each strain in 6 PD and one hemp seed on each block, keep blocks well apart and add water to cover bottom of dish; -9, in the same manner make 1 control PD of "♀" × "♀" and and another of "♂" × "♂"; -5, move blocks together so that mycelia are in good contact. Male or female gametangia are not necessarily limited to one strain or the other and gametangia not infrequently appear in the

minutes later, at the instant when the vesicle ruptured and the zoospores are scattering. 7 (×750). *Pythium oligandrum.* Oogonium with its conspicuously spiny wall. 8 (×750). *Phytophthora drechsleri.* Oogonium and amphigynous antheridium.

unmated control plates. However, mating of the two strains usually markedly stimulates gametangium formation. (b) An alternative mating that has also provided interesting material is *Ph. cactorum* (Lebert and Cohn) Schroeter (51-22) × *Ph. citrophthora* (Smith and Smith) Leonian (51-13). The preparation is just as above but with slightly different timing, *viz.:* -16, start pd of BBL, -12, start water cultures in PD, -5, move mycelia into contact. In this cross gametangium formation is again variable, and both amphigynous and paragynous antheridia



FIGS. IX, 1-6. Peronosporales and zoospores from three other orders. 1 (× 375). *Phytophthora citrophthora.* Typical mycelium and papillate zoosporangia. 2, 3. *Phytophthora* sp. 47-1. 2 (× 375). Proliferation of a hypha through an old sporangium to produce a new one above. 3 (× 750). A group of four "nested" sporangia with a fifth one proliferating within. 4-6 (× 750). Zoospores fixed in osmic acid fumes. 4. *Rozella allomycis,* stained with gentian violet. 5. *Allomyces macrogynus,* not stained. 6. *Sapromyces elongatus,* not stained.

are produced in varying proportions apparently related to slight changes in cultural conditions. *Ph. citrophthora* also forms a fine crop of sporangia as shown in FIG. IX, 1.

CONCLUSIONS

For ten years students at Berkeley have made their own observations of the fascinating living fungi shown in the figures you have just seen. Once again the two foundations of our scheme of organization should be emphasized. First is the use of pure cultures, for only with these can we eliminate unknown variables and count upon the duplicability of the material. Second is the demonstrated fact that, given an environment which can be controlled, predictable responses can be induced at will time and time again.

I have written about the water molds because I know them best and I think they are most in need of reemphasis at the present time. Laboratory preparations for the rest of the course are organized insofar as possible in the same manner up into the terrestrial Phycomycetes and on through representative ascomycetous genera. Eventually it should be of great value to extend this sort of organization to cover all groups of fungi. It is my hope that other investigators and teachers will contribute their special knowledge and write additional chapters in what could be termed a laboratory guide or *vade mecum* for dynamic mycology. Those who do will find their efforts doubly rewarded for, in the course of their labors, they will uncover a host of basic and exciting problems that underscore the age-old and essential unity between teaching and research.

In our Society then, as in our teaching, let us strive always to foster and develop the widest possible approach to mycology. Let us see to it that every aspect of our mycological organization reflects this breadth of approach, this understanding, and this appreciation of the many facets of research on the fungi, and the strength that comes from visualizing mycology as one of the great biological disciplines.

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NUCLEAR BEHAVIOR IN MYCELIUM OF A SOLOPATHOGENIC LINE AND IN A CROSS OF TWO HAPLOID LINES OF USTILAGO MAYDIS (DC.) CDA.[1]

HOWARD G. EHRLICH [2]

(WITH 1 FIGURE)

The investigation reported here was undertaken to compare the nuclear behavior of a solopathogenic line of *Ustilago maydis* (DC.) Cda. with that of two sexually compatible haploid lines during vegetative growth and chlamydospore formation.

MATERIALS AND METHODS

The solopathogenic line *410qq* and the haploid lines $10A_4$ and $17D_4$ (the parent lines of *410qq*) were grown separately on potato-dextrose agar slants and in stationary as well as continuously agitated potato-dextrose broth. At various times smears from these cultures were prepared for cytological examination.

Seedlings of *Zea mays* L. 'Marcross' were inoculated with either *410qq* or with a mixture of $10A_4$ and $17D_4$ from the agitated potato-dextrose-broth cultures, using the vacuum technique of Rowell and DeVay (**1953**). Seedlings were harvested at intervals beginning four days after inoculation until their death. The material was prepared for nuclear analysis of the mycelium via the paraffin technique. The most satisfactory staining results were obtained when basic fuchsin (DeLamater, **1948**) with a counterstain of fast green and picric acid in clove oil was employed in conjunction with four percent formaldehyde as a fixative for the vegetative cells from broth cultures and with F.A.A. as a fixative for mycelium in the host.

[1] The basic laboratory work was done in the Department of Plant Pathology and Botany, University of Minnesota, St. Paul. Paper No. 3893, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul 1, Minnesota.

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RESULTS AND DISCUSSION

The cells of each of the three lines when grown on artificial media were typically uninucleate.

The number of mycelial cells, in inoculated host material, analyzed for nuclear number is given in TABLE I, and the percentage of uni-

TABLE I

NUMBER OF MYCELIAL CELLS OF USTILAGO MAYDIS ANALYZED, IN THE HOST, FOR NUCLEAR NUMBER

Isolate or Cross	Number of cells analyzed										
	Days after inoculation										
	4	6	8	11	13	15	18	21	23	30	34
410qq	11	40	76	583	800	800	800	800	900	800	800
$10A_4 \times 17D_4$	104	87	712	776	833	818	1073	1028	1013	—	—

nucleate, binucleate, and multinucleate cells is shown in FIG. 1. The low number of cells counted in the early samples (to 11 days in *410qq* and 8 days in $10A_4 \times 17D_4$) represents the total analyzable number found in an examination of the major portion of each of five seedlings. Subsequent cell totals represent arbitrary stopping points, since it became apparent that additional counting would not significantly alter the data. Therefore, the data also reflect the relative initial growth rate of the solopathogen and the cross in the host.

Cells of the solopathogenic line in the host were characteristically uninucleate throughout vegetative growth and chlamydospore formation, although a few binucleate cells were found (FIG. 1; cf. also Christensen, **1931**).

Nearly all (99%) of the mycelial cells resulting from the cross $10A_4 \times 17D_4$ were uninucleate four days after inoculation. Eleven days after inoculation 67% of the cells were binucleate, and later the cells became predominantly (90%) uninucleate again. A few cells from the cross were multinucleate (three to six nuclei per cell) throughout the period of study (FIG. 1).

Sleumer (**1932**) similarly reported the first occurrence of binucleate mycelium 2–4 days after inoculation and stated that at 5–6 days binucleate mycelium predominated. He further reported that the binucleate condition was not uniformly present and did not last. Christensen (**1931**) also reported uninucleate, binucleate, and multinucleate cells,

and Hanna (**1929**) illustrated early dikaryotization. These observations of early mycelial nuclear condition do not agree with those of Lutman (**1910**), Rawitscher (**1912**), and Seyfert (**1927**). Christensen (**1931**) expressed the opinion that karyogamy usually occurs before gelatinization and segmentation of the mycelium, with Rawitscher (**1912**) thought it occurred afterward. Sleumer (**1932**) indicated that karyogamy may

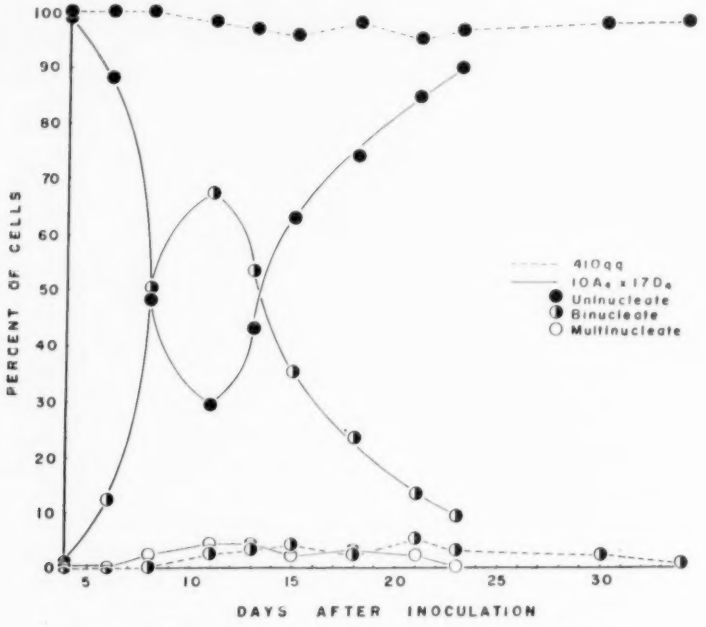

FIG. 1. The nuclear behavior of *Ustilago maydis* in the host.

occur at varying times in the life cycle. He described one- and two-nucleate mycelial cells, the single nucleus being larger than either of those in binucleate cells, and suggested that the large, single nucleus may result from karyogamy. These observations by Sleumer were confirmed in the present investigation. Uninucleate cells with large nuclei were first noted 11 days after corn was inoculated with the crossed lines and accounted for the major portion of uninucleate cells subsequently found. The continued presence of some uninucleate mycelium with small nuclei was regarded as continued growth of the clonal lines in which dikaryotization had not, as yet, occurred. The cells of segmented

hyphae as well as young and mature chlamydospores observed in the present investigation were characteristically uninucleate and only rarely binucleate (cf. also Christensen, **1931**; Sleumer, **1932**). Uninucleate mycelium was associated with the areas of the gall in which the chlamydospores were formed.

Therefore, the initial increase in the binucleate condition previously mentioned is considered evidence of dikaryotization, and the later decrease in the binucleate condition and consequent increase in the uninucleate is interpreted as evidence of nuclear unions. Thus the union of nuclei in the cross occurred prior to the recognizable formation of chlamydospores, which in these experiments were formed about 15 days after inoculation. The appearance of the galls in no way suggested the presence of the first-formed chlamydospores. The chlamydospores were found only after microscopic examination of a large amount of sectioned material. Chlamydospores developed about the same time in the solopathogen, but no indications of nuclear union were found.

The number of nuclei per cell of the solopathogen ranged from one to two. In the cross the range was from one to six nuclei per cell. Since the frequency of two nuclei per cell in the solopathogen and three to six nuclei per cell in the cross was very low (cf. FIG. 1) and no characteristic relation between multinucleate cells and the segmentation stage as reported by Lutman (**1910**) was observed, the multinucleate condition is considered due to mitoses involved in normal mycelial growth.

Observations indicate, further, that the two nuclei in approximately 85 percent of the dikaryotic fungus cells resulting from the cross in the host differ in size. Since all the nuclei were relatively small, measurement with the ocular micrometer involved some degree of error. However, a measurement of 25 nuclei in each case substantiated observable size differences. The larger nucleus of the dikaryotic cell ranged from 2.5 μ to 1.7 μ, 1.7 μ being most common; and the size of the smaller nucleus was 2.1 μ to 1.1 μ, 1.2 μ being most frequent. The nuclei of the isolate $10A_4$ when grown alone in artificial media ranged from 2.1 μ to 1.7 μ, and $17D_4$ nuclei were 1.7 μ to 1.1 μ in size. Thus, the size of $10A_4$ nuclei was similar to the larger nucleus of the dikaryotic cell, and $17D_4$ nuclei were similar in size to the smaller nucleus. Nuclei of the solopathogen *410qq* in the host as well as in artificial culture ranged from 2.5 μ to 1.7 μ, the same as the larger nucleus of the dikaryotic cells arising from the cross $10A_4 \times 17D_4$. The most frequent size of these *410qq* nuclei was 2.5 μ, however. The size of nuclei in the chlamydospores of both the solopathogen and crossed isolates was the same, i.e.,

2.5 μ to 3 μ. Where binucleate chlamydospores were found, both nuclei had the same dimensions, and each was about the same size as the nucleus of the uninucleate chlamydospores. The latter suggests that some chlamydospores may contain two diploid nuclei (cf. also Christensen, **1931**). Conceivably, one or both of the nuclei could undergo meiosis and genetic segregation upon chlamydospore germination.

Chromosomes of *U. maydis* were observed, but the exact number was not definitely established. The present investigation neither confirms nor excludes, on a cytological basis, dissociation or vegetative segregation in *U. maydis*.

SUMMARY AND CONCLUSIONS

1) The three isolates of *U. maydis* investigated were typically uninucleate in artificial culture.

2) The solopathogen in the host was also characteristically uninucleate throughout its development, and no suggestion of karyogamy was found.

3) Mycelium resulting from the cross of compatible haploid lines in the host was initially uninucleate. However, a progressive increase in the percentage of dikaryotic cells occurred until 11 days after inoculation at which time the trend reversed and uninucleate cells again predominated. The changes in nuclear condition were interpreted as being due to dikaryotization followed by nuclear unions. Karyogamy occurred prior to recognizable chlamydospore formation.

4) Significant phases of nuclear behavior in the life cycle may be missed if sampling is not sufficiently early, late and frequent.

ACKNOWLEDGMENTS

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TWO NEW FIFTY-YEAR-OLD SPECIES OF MYXOBACTERIA

JOHN E. PETERSON

Roland Thaxter erected the Order Myxobacteriales of the Schizomycetes in a paper published in the *Botanical Gazette* in 1892. He followed that initial paper with three others (**1893, 1897, 1904**) in the same journal; that of 1904 was his last concerning the myxobacteria.

Included in Thaxter's collection of myxobacteria, now in possession of the Farlow Herbarium, Harvard University, are two collections dated after 1904. One of these, dated 1905, is designated as a type; the other, dated 1907, is labeled as a new species. No reference to either of these myxobacteria appears in the literature. Thaxter apparently added these specimens to his collection of myxobacteria, but failed to publish descriptions of them. The two organisms have not been described by any other worker in the intervening fifty years.

Sorangium aurantiacum Thaxter ex. Peterson

Synonymy: *Polyangium aurantiacum* Thaxter; unpublished, but the original designation given the collection.

TYPE material: On rat dung; collected at Sandy Run, South Carolina, by R. Thaxter in March, 1905; determined by R. Thaxter; bearing Thaxter's accession no. 4569 and designated by Thaxter as *Polyangium aurantiacum* Type; on deposit in the Farlow Herbarium.

Fruiting body an irregular mass, generally an appressed cushion; ranging in size up to 350–400 μ by 100 μ high; orange-brown in reflected light, bright orange in transmitted light. Fruiting body composed of polygonal, angular cysts, from 4.5 μ in diameter up to 10–13 μ, the majority about 7–8 μ in size. Each cyst bounded by a distinct, bright yellow wall about 1 μ thick; wall showing no affinity for aniline-blue dye, but dye readily diffuses through to stain interior. Encysted rods only faintly discernible; about one dozen such rods in smallest cysts, 2–3 dozen in larger cysts; not closely packed together; about 1 × 3–4 μ. Vegetative cells not described.

The material on which this description is based consists of a single collection of several small pieces of plant material, some rat dung pellets

and some pieces of unidentifiable material. Several dozen fruiting bodies are scattered over most of this substrate material. A notation by Thaxter on the specimen box indicates that this material had been placed in a moist-chamber culture. This procedure, in all probability, gave rise to the myxobacterial fruiting bodies.

The above description, of course, is based on material fifty years old. It is entirely possible that age has effected changes in the specimens. However, observations of many other species of myxobacteria of similar age, compared with those which are fresh, have convinced the writer that myxobacterial fruiting bodies, in general, preserve well with little change other than occasional color fading. In consideration of the specific epithet chosen by Thaxter, the color has probably not changed appreciably in this case.

As is true of all of the other members of the genus *Sorangium*, the fruiting bodies of *S. aurantiacum* are nothing more than irregularly-shaped cushions which defy further description. A mounted fruiting body, observed under low magnifications, is clearly seen to be composed of tiny, angular cysts. No larger divisions, such as the primary cysts of some of the species of *Sorangium*, are present. No bounding membrane surrounding the entire fruiting body is discernible, although the fruiting body may be easily mounted intact. One must actually press the cover slip quite hard to squash the fruiting body.

Under low magnifications, the mounted fruiting bodies are bright yellow in color. The yellow pigmentation appears to be primarily confined to the thick cyst walls, and it is extremely persistent in aniline-blue mounts. The encysted rods within the cysts are impossible to see in unstained preparations and very difficult to see in stained mounts. For this reason, the determined size of 1×3–$4\ \mu$ and the stated number of encysted rods per cyst may be open to some question. These determinations are as accurate as they can be made, however.

Only three genera of myxobacteria, *Polyangium*, *Chondromyces* and *Myxococcus*, were recognized in 1905. Jahn (**1924**) erected the genus *Sorangium* to include those members of the genus *Polyangium* which possessed fruiting bodies composed of small, polygonal cysts. There is no question but that Thaxter's *Polyangium aurantiacum* is actually a member of the genus *Sorangium* as that genus is interpreted today. This organism will be referred to as *Sorangium aurantiacum* Thaxter ex Peterson hereafter in this paper.

Previous to the present description of *Sorangium aurantiacum*, eight species of *Sorangium* have been described. Two of these, *S. nigrum* Krzem. & Krzem. (**1937**) and *S. nigrescens* Krzem. & Krzem. (**1937**),

are black in color and can be immediately excluded from further comparisons with *S. aurantiacum*. Two others, *S. septatum* (Thaxter) Jahn (**1904**) and *S. compositum* (Thaxter) Jahn (**1904**), produce both secondary and primary cysts and may be easily excluded from further consideration.

The fifth species of *Sorangium*, *S. sorediatum* (Thaxter) Jahn, was described by Thaxter in 1904. No specimens of this species are included in Thaxter's collection now in the Farlow Herbarium and the description of the organism is sketchy. However, since Thaxter collected and studied both species in question, it seems logical to accept his opinion that the two are different. The author has found an organism on the bark of living trees which he has identified as *S. sorediatum*. This organism is obviously different from *S. aurantiacum*.

The description of the sixth species of the genus *Sorangium*, *S. schroeteri* Jahn (**1924**), indicates only minor differences between this species and *S. aurantiacum*. A slide of mounted *S. schroeteri* fruiting bodies was sent to Thaxter by Finkenkrug in Germany, and it is currently in Thaxter's collection in the Farlow Herbarium. The author has examined and photographed the specimens on this slide and he believes that they are quite different from *S. aurantiacum*.

The author has never seen the seventh species of *Sorangium*, *S. spumosum* Krzem. & Krzem., but it appears to be quite different from any of the other species of the genus on the basis of the Krzemieniewskys' description and photograph (**1927**). The cysts of *S. spumosum* are spherical or oval instead of polygonal, they are usually grouped in single or double rows instead of in huge masses, and the walls of the cysts are brownish or hyaline and transparent instead of bright yellow. There seems to be no question but that *S. spumosum* and *S. aurantiacum* are quite distinct species.

The final species of the genus *S. cellulosum* Imsenecki & Solntzeva (**1937**), is also unknown to the author. However, the description of the species indicates striking differences between *S. cellulosum* and *S. aurantiacum*. The cysts of *S. cellulosum* are much smaller than those of *S. aurantiacum*, the color of the fruiting body of *S. cellulosum* is rusty-brown as opposed to the orange-brown fruiting body of *S. aurantiacum* and there is no discernible wall around the cysts of *S. cellulosum*, while the cyst walls of *S. aurantiacum* are striking.

The writer has studied five of the eight described species of *Sorangium*. None of these five species is the same as Thaxter's undescribed *Sorangium aurantiacum*. The three species which the author has not observed can all be eliminated from consideration on the basis of rather

obvious differences. *S. sorediatum* and *S. schroeteri* are most similar to *S. aurantiacum*. Thaxter was familiar with both of these species and still designated *S. aurantiacum* as a new species. In addition, the author has examined limited material of both of these species and he believes them to be different from *S. aurantiacum*. *Sorangium aurantiacum* is an undescribed species of the order Myxobacteriales even though it is fifty years old.

Myxococcus lacteus Thaxter ex. Peterson

Synonymy: *Chondromyces lacteus* Thaxter; unpublished, but the original designation given the organism.

TYPE material: On bark; collected at Chocorua, New Hampshire, by W. G. Farlow in 1907; determined by R. Thaxter; bearing Thaxter's accession no. 4562 and designated by Thaxter as *Chondromyces lacteus* n. sp.; on deposit in the Farlow Herbarium.

Fruiting body a single, globose to pear-shaped mass of shortened rods (spores) on a centrally-attached stipe. Spore mass milky-white, opaque. Stipe crystalline, hyaline, seldom opaque. Spore mass 50–150 μ in diameter; stipe usually as long as the diameter of the spore mass by 10–25 μ wide; total height of fruiting body 100–300 μ. No membrane discernible around spore mass. Stipe completely hyaline in transmitted light; delicately, but distinctly, striated longitudinally; no cells of any type included; continuing about half-way up into spore mass to produce a columella effect; highly resistant to aniline-blue dye. Spores spherical, 1 μ or less in diameter; little affinity for aniline-blue dye; somewhat refractile, even when stained. Vegetative cells not described.

The single collection on which the above description is based consists of five small pieces of bark over which many dozen fruiting bodies are scattered. As stated in the description, the color of the spore-mass portion of the fruiting body is milky-white and opaque. On the basis of the specific epithet which Thaxter selected, it may be assumed that this is the true color. Some fruiting bodies in which the spore mass is crystalline may be found. However, these are much in the minority and it is the writer's opinion that these are atypical, inadequately-matured fruiting bodies.

No membrane of any type is discernible around the spore mass, but a definite edge of the spore mass is apparent. The spore mass is very persistent and can be easily mounted without breaking up. In fact,

actual pressure on the cover slip is necessary before mounted fruiting bodies can be broken up.

There is no doubt that this collection represents a species of the genus *Myxococcus*. The author finds it impossible to conjecture how Thaxter could designate the organism as a *Chondromyces* species unless he failed to mount specimens and to observe them microscopically. Thaxter was well aware of the characteristics of *Myxococcus*, since he erected the genus himself (**1892**).

Only two of the eleven described species of *Myxococcus* are stipitate. One of these two, *M. ovalisporus* Krzem. & Krzem. (**1926**), possesses a poorly developed stipe, which contains cells and oval-shaped spores and is light yellow in color. The writer has never examined this organism, but, on the basis of the description, *M. ovalisporus* must be quite distinct from *M. lacteus*.

The remaining stipitate *Myxococcus* species, *M. stipitatus* Thaxter (**1897**), was described by Thaxter, although the description is sketchy. It is reasonable to assume, therefore, that Thaxter felt the two species in question were quite distinct. The writer has isolated and studied what he considers to be *M. stipitatus* and he is of the opinion that *M. stipitatus* and *M. lacteus* are quite different species. The stipe of *M. stipitatus* is typically very wide in proportion to the spore mass and the spore mass is typically globose. The spore mass of *M. lacteus* is proportionately much wider than the stipe and it is typically conical, or pear-shaped. The stipes of *M. stipitatus* fruiting bodies are not striated as are those of *M. lacteus*. There is no columella in *M. stipitatus* as there is in *M. lacteus*. The spores of *M. stipitatus* are somewhat larger than those of *M. lacteus*, but, more important, they have a high affinity for aniline-blue dye and stain deep blue, while those of *M. lacteus* have almost no affinity for aniline blue. As is true of *Sorangium aurantiacum*, *Myxococcus lacteus* is an undescribed species of the order Myxobacteriales, even though it is fifty-one years old.

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SOME LEAFSPOT FUNGI ON KANSAS GRAMINEAE[1]

RODERICK SPRAGUE[2] AND CLARK T. ROGERSON[3]

(WITH 2 FIGURES)

The following new or noteworthy fungi were collected by Rogerson and associates in 1956 and 1957 in widely scattered places in eastern Kansas.

Rhynchosporina tridentis sp. nov.

Maculis linearibus, margine rubri-vinaceis, centro pallidis 0.5–1 × 1–2 mm diam., acervulis obscuris, hyalinis, intra- et sub-epidermalibus v. cuticularibus et substomaticis; conidiophoris brevibus, cylindraceis, 3–5 × 1–1.3 μ; sporulis hyalinis, falcatis, aseptatis, 12–15 × 1.8–2.1 μ.

Spots linear with red-wine-colored margins and pale buff, straw-colored or isabelline centers, small, 0.5–1 × 1–2 mm diam. or appearing larger by confluence, delimited by the leaf veins; pseudo-acervuli obscure, consisting of intercellular and intracellular hyaline hyphae aggregated to some extent in the subepidermal area but penetrating into the substomatal, stomatal and cuticle areas, sending short, cylindrical, peg-like conidiophores about 3–5 × 1–1.3 μ diam. through the cuticle and stomatal areas; spores hyaline, strongly curved, pointed at each end (falcate), aseptate, 12–15 × 1.8–2.1 μ, mostly 13 × 2 μ; spores accumulated in loose groups above the stomata in most instances.

Hab. in living leaves of *Tridens flavus* along Wildcat Creek, near Manhattan, Kans., leg. *C. T. Rogerson*, July 28, 1956. TYPE is Kans. State Coll. ex myc. Herb. *R 3781* (WSP 42831). Paratypes: near Lawrence, Kans., KSC *R 3810* (WSP 42967); West Manhattan, Kans. KSC *R 3808* (WSP 42968); Atchison, Kans. *R 3809* (WSP 42969).

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In Von Arx's definitive study of *Glocosporium* Desm. et Mont. (**1**) he found that the type of the genus was identical with *Marssonina populi* (Lib.) Magn. The name *Marssonina* Magn. is a synonym therefore of *Glocosporium* Desm. et Mont. However, the popular concept of *Glocosporium* sensu Saccardo (**3**) is a complex of fungi assignable, according to Von Arx, to a number of genera in the Melanconiales, Moniliales and even Sphaeropsidales, among others. Von Arx recommended that *Marssonina* be conserved and *Glocosporium* sensu Saccardo rejected. Actually, after Von Arx had removed all fungi not belonging in *Glocosporium* sensu Saccardo there was very little left, apparently, except a number of forms which differed from *Colletotrichum* only in the absence of setae. He apparently considers the presence or absence of setae of less than specific significance. At least he assigns 288 species to synonomy under *Colletotrichum glocosporioides* Penz. and many of these lack setae.

Among the genera employed by Von Arx to dispose of *Glocosporium*-like forms is *Rhynchosporina* Von Arx which differs from *Rhynchosporium* in the absence of septations in the spores. He bases this on *Glocosporium meinersii* Sprague whose spores arise from peg-like sterigmata which push through the epidermis. The acervuli are said to be atypical. Actually they scarcely exist as more than loosely aggregated stromatic tissue. The same may be said for the fungus on *Tridens*. The mycelia twine about through the interior of the leaf sometimes forming loose "pseudo-acervuli" in the vicinity of stoma but working into the cuticular area where erect simple or rarely branched short pegs or conidiophores push to the surface (FIG. 1, A). Sometimes the cuticle tends to shrink or partially disappear.

The conidiophores or pegs are sometimes almost palisade-like in abundance, other times rare. They average about 3–5 × 1 μ in stained material. Spores are found in small groups often clustered around stomata. This fungus is very difficult to study and is not easy to see in prepared slides. It stains more or less readily with phloxine. On the basis of detailed study of many prepared slides as well as of fresh material, the fungus is placed in *Rhynchosporina* V. ARX. Because of the presence of pseudo-acervuli, if this term may be used, perhaps the fungus is closer to the Melanconiales than the Moniliales. It could be placed in either.

In culture this fungus produces a pink to red mucous growth somewhat resembling *Glocosporium bolleyi* Sprague. The latter is called *Aureobasidium bolleyi* (Sprague) Von Arx (**1**). Since *A. bolleyi* is known only from pure culture its exact status is still uncertain. The

spores of *A. bolleyi* differ markedly from those of *R. tridentis*. In culture *R. tridentis* forms spores in clusters of several on the end of short lateral branches.

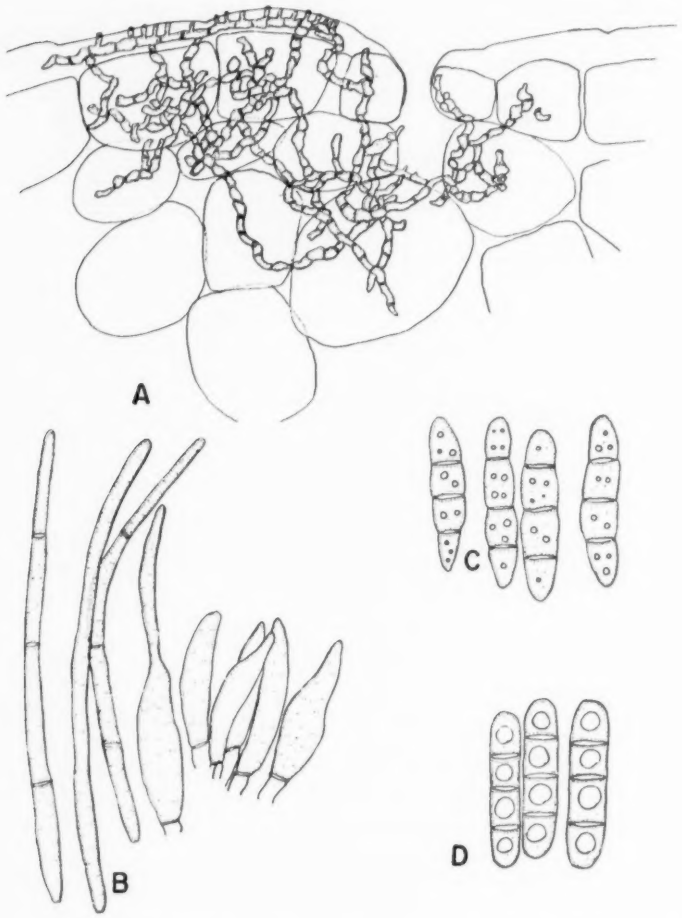

FIG. 1, A. *Rhynchosporina tridentis* showing development of mycelium in host, type. B. Spores and conidiophores of *Cercosporella boutelouae*, type. C. Pycnidiospores of *Stagnospora subchlorinoflavida*, type. D. Pycnidiospores of *Stagnospora tridentis*, type. Drawn with the aid of a camera lucida × 1000.

Cylindrosporium campicola sp. nov. Rogerson and Sprague

Maculis fuscis, linearibus, margine rubi-vinaceis, diffusis, centro massa albida, lineari; conidiophoris ad instar valli instructis; hyalinis, cylindraceis v. paliformibus, apicibus acutis, 15–20 × 4–5 μ; conidiis hyalinis, rectis, curvulis, flexuosis, filiformibus, basi obtusis, apicibus acutis, 0–3-septatis, 50–77 × 2–3 μ.

Spots fuscous, linear, often only 2–3 mm long, margin reddish-vinaceous or sometimes fawn-colored, sometimes somewhat diffuse, center of lesions showing by eruption a narrow, dry, white spore mass; conidiophores in a palisade-like layer, hyaline, emerging from the leaf tissue by eruption of the outer cell layers, cylindrical but the apex pointed, therefore somewhat stake-shaped, 15–20 × 4–5 μ; conidia hyaline, straight, curved or sometimes recurved, filiform but base obtuse and apex acute, 0–3-septate, 50–77 × 2–3 μ.

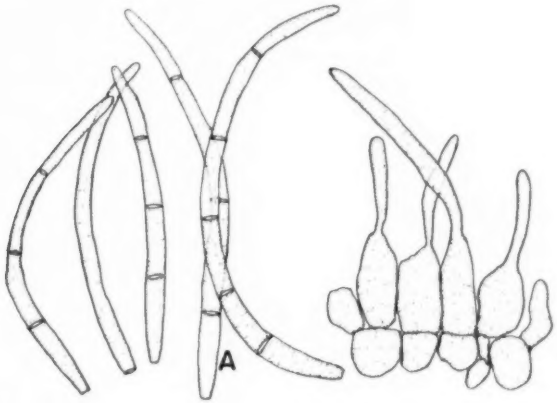

FIG. 2, A. Spores and conidiophores of *Cylindrosporium campicola*, type. Drawn with the aid of a camera lucida × 1000.

Hab. in living leaves of *Cynodon dactylon*, leg. *Henry Wolfe*, Hutchinson, Kans., June 5, 1956, ex myc. Herb. KSC *R 3782* (WSP 42832), TYPE. Paratypes: on *C. dactylon*, leg. *C. T. Rogerson*, Manhattan, Kans. KSC *R 3804* (WSP 42964), Oct. 17, 1957; on *Chloris verticillata*, leg. *C. T. Rogerson*, near Spring Creek, Fostoria, Kans. KSC *R 3805* (WSP 42963), July 16, 1957.

The senior author assigned this material at one time to *Glococercospora sorghi* D. Bain and Edg. (**6**). More and, perhaps, better material of *Cylindrosporium campicola* plus additional study now indicates that this fungus is closer to the Melanconiales than to the Moniliales. *Gloco-*

cercospora sorghi forms tiny orange sporodochia which are scattered over the leaf surface. The conidiophores of *G. sorghi* are obscure, short, scarcely more than 5 μ, sometimes up to 10 μ long. Since *C. campicola* erupts through narrow shallow slits with definite conidiophores (FIG. 2, A) it is now placed in *Cylindrosporium*. It differs from *C. andropogoni* Sprague and Rogerson in having much larger spores with more septations. The specific name of *C. campicola* refers to its preference for a prairie habitat.

The holotype material on *Cynodon* is not distinguishable from the paratype of *Chloris*. The spores on the former measure 50–77 × 2–3 μ, those on *Chloris*, 50–70 × 2–3 μ.

Cercosporella boutelouae sp. nov.

Maculis linearibus, 1–4 × 0.2–0.4 mm diam., fuscis, margine angusto, rubri-vinaceo; myceliis chlorinis v. hyalinis in parenchymaticis cellulis, aggregatis prope epidermidem; conidiophoris aggregatis, lagunculiformibus, apicibus subacutis, 10–15 × 4–5 μ, hyalinis; conidiis hyalinis, curvulis, basibus obtusis, apicibus acutis, 0–5-septatis, 43–50 × 2.5–3.0 μ.

Spots linear, 1–4 × 0.2–0.4 mm diam., fuscous, margin obscure, narrow, reddish wine-colored, mycelia hyaline to yellowish or brownish where aggregated, growing in parenchyma tissue sometimes completely around the prominent woody veins, aggregating near the surface and forming layers of conidiophores on the leaf surface, conidiophores bottle-shaped, inconspicuous, sometimes sub-acute at the tip, hyaline, 10–15 × 4–5 μ; conidia hyaline, curved, especially abruptly curved about two-thirds of the distance from the slightly enlarged obtuse base, apex acute, 0- to 5-septate, 43–50 × 2.5–3.0 μ.

Hab. in living leaves of *Bouteloua curtipendula* in prairie about five miles west of Latham, Butler Co., Kans., leg. *C. T. Rogerson*, ex myc. Herb. KSC *R 3806* (WSP 42965), July 26, 1957, TYPE. Paratype: Five miles south of Manhattan, Kans., leg. *C. T. Rogerson*, KSC *R 3807* (WSP 42966), Sept. 16, 1957.

This is placed in the Moniliales because there is no evidence of a true acervulus. Although superficially it resembles *Cylindrosporium campicola*, *C. boutelouae* is definitely hyphomycetous. Since *Cercosporella* was originally described as a hyaline-spored companion of *Cercospora*, the presence of well defined conidiophores in our fungus (FIG. 1, B) does not exclude it from *Cercosporella* (3).

In the field *C. boutelouae* could be easily confused with *Cercospora boutelouae* Chupp and Greene but they are, of course, microscopically different.

There are some pycnidia of *Stagonospora* sp. in the type of *C. boutelouae*. They are referred to a new species described later in this paper.

CERCOSPORA MUHLENBERGIAE Atk. Collected on *Muhlenbergia* sp. along a roadside northeast of Williamstown, Jefferson Co., Kans., July 19, 1957 (KSC *R 3813;* WSP 42971). The spores were 3–5-septate. Chupp (**2**) states that "this (*C. muhlenbergiae*) has mostly one-septate conidia, therefore is not a *Cercospora*." The material from Kansas which has multiseptate spores seems to belong in *C. muhlenbergiae* and would appear to be referable to *Cercospora*. Some modification of the description of this species may be desirable in order to include the range of septations present in some material. Some material collected later in the season (Oct. 12, 1957) on *M. schreberi* Gmel. at Manhattan showed typical symptoms but almost no spores. The few spores of this species present in the specimen are like the ones mentioned by Chupp. Most of the spores in this material are of other fungi associated with the *Cercospora*, including *Fusarium* sp. and *Curvularia geniculata* (Tr. and Earle) Boed. (KSC *R 3834*).

Phyllosticta andropogonivora sp. nov.

Maculis rubescentibus v. brunneis, centro pallidis, sublinearibus; pycnidiis paucis, globosis v. lenticulari-globosis, nigris, erumpentibus, ostiolatis, 70–110 × 60–95 μ, pycnosporulis capsularibus, utrinque obtusiusculis, 2-guttulatis, hyalinis, aseptatis, 14–15 × 4–5 μ.

Spots reddish to brown, tardily pale in center, sub-linear to somewhat diffuse by fusion; pycnidia few, often only one in some spots, black, globose to somewhat elliptical, erumpent, ostiolate, finally eroding apically, 70–110 × 60–95 μ; pycnidiospores capsular with obtuse ends and with a prominent oil globule at each end, aseptate, 14–15 × 4–5 μ.

Hab. in living leaves of *Andropogon gerardi*, Beagle, Miami Co., Kans., leg. *C. T. Rogerson*, June 10, 1957, ex myc. Herb. KSC *R 3815* (WSP 42962), TYPE.

In mounts from older lesions in the type we encountered an immature perithecial form with short club-shaped asci and yellow hyaline contents, too immature to determine.

The above described *Phyllosticta* is different from any other of this genus known to us on Gramineae. It is, perhaps, closest to *P. healdii* Sprague but lacks the abundant linearly arranged pycnidia of that species. *P. andropogonivora* is characterized by its capsule-shape spores with the prominent oil drops at each end.

Stagonospora tridentis sp. nov.

Maculis ellipticis, isabellinis, sanguineo-marginatis, pycnidiis nigris, pseudoparenchymaticis, ostiolatis, subglobosis, 71–120 × 50–85 μ; pycnosporulis hyalinis, cylindraceis, utrinque obtusiusculis, (2–)3-septatis, guttulatis prominulis, 22–25 × 5.4–6.1 μ.

Spots elliptical, isabelline, margin reddish, pycnidia black, pseudoparenchymatous, subglobose, ostiolate, sometimes widely so, 71–120 × 50–85 μ; pycnidiospores hyaline, cylindrical, both ends blunt, (2–)3-septate, each cell with a large, shiny guttule, 22–25 × 5.4–6.1 μ.

Hab. in living leaves of *Tridens flavus*, along Sand Creek near St. George, Pottawatomie Co., Kans., leg. *C. T. Rogerson*, ex myc. Herb. KSC *R 3811* (WSP 42961), TYPE.

This fungus is characterized by its straight-sided, capsule-shaped spores with blunt ends and large shiny guttulae which occur in each cell (FIG. 1, D). In size and shape of spore this fungus differs from *St. simplicior* Sacc. and Berl., *St. subseriata* (Desm.) Sacc. and *St. paspali* Atk.

Stagonospora subchlorinoflavida sp. nov.

Maculis linearibus v. sub-rectangularibus, variabilibus, brunneo-fuscidis, centro pallescentibus; pycnidiis nigris, pseudoparenchymaticis, globosis, paucis v. subgregariis, 60–100 × 45–85 μ, ostiolatis; pycnidiosporulis fusiformibus, sub-chlorinoflavidis, triseptatis, 20–25 × 3.8–5.6 μ.

Spots linear to sometimes almost rectangular, variable, brown-fuscous but becoming paler, almost isabelline in the centers of older lesions, pycnidia black, depressed-globose, few, or sometimes crowded in lines, 60–100 × 45–85 μ, ostiolate; pycnidiospores fusiform, somewhat constricted at the septa, contents coarse, greenish-yellow, hyaline, with several oil drops in each cell, mostly tri-septate, 20–25 × 3.8–5.6 μ.

Hab. in living leaves of *Bouteloua curtipendula* on prairie five miles west of Latham, Butler Co., Kans., Sept. 27, 1957, leg. *C. T. Rogerson* ex myc. Herb. KSC *R 3812* (WSP 42970), TYPE. Also in type of *Cercosporella boutelouae* (KSC *R 3806*).

This fungus with its faintly tinted and lumpy fusiform spores (FIG. 1, C) has some resemblance to a fungus on *Andropogon* spp. from Oklahoma which has been called *St. ischaemi* Sacc. (**5**, Fig. 57, B). The spores of the Oklahoma material measured 12–18 × 2.8–4.1 μ; those of the type of *St. ischaemi* measured 12 × 4 μ. Neither the Kansas material nor the Oklahoma fungus is very close to *St. ischaemi*. Therefore

St. subchlorinoflavida is not referable to any known *Stagonospora* that has been noted. The Oklahoma material needs restudy.

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SOME NEW DISCOMYCETES FROM CALIFORNIA

EDITH K. CASH [1]

(WITH 10 FIGURES)

Over a period of many years the writer has had the opportunity of studying the numerous collections of Discomycetes made by Harold E. Parks in the vicinity of Trinidad and at various other localities in California. Few fungi however inconspicuous escape Mr. Parks's notice, and many new records are due to his observations. Additional California specimens were examined through the kindness of Lee Bonar of the University of California and his associates. Several collections made by the late C. L. Shear in 1939 are also discussed here. Observations on some of these fungi have been published previously (**1**, **2**); those still undescribed, so far as can be ascertained, are named in this paper. Type specimens are deposited in the National Fungus Collections, Beltsville, Maryland, and duplicates in the herbarium of the University of California at Berkeley.

1. **Cistella parksii** sp. nov. (FIG. 1)

Apothecia dispersa, ceracea, 0.1–0.3 mm. in diametro et altitudine, turbinata usque cupulata, ad basim fuscum brevem crassum contracta, pellucido-alba usque cremeo-ochracea, margine subfimbriato; asci fusoideo-clavati, ad apices subattenuati et rotundati, breviter stipitati, poro iodi ope caerulescente, octospori, 25–40 × 4–6 μ; ascosporae 1–2-seriatae, hyalinae, unicellulares, guttulatae, clavato-ellipsoideae vel clavato-fusoideae, 4.5–7 × 1.5–2.5 μ; paraphyses filamentosae, hyalinae, ascos superantes, apicibus subinflatis; excipulum prosenchymaticum, ad basim fuscum, marginem versus hyalinum, ex hyphis tenuibus subclavatis, ad apices 2–3 μ et minute verrucosis compositum.

Apothecia sparse, soft-waxy, 0.1–0.3 mm in diameter and height, subsessile, turbinate to cupulate, narrowed below to a short, thick, dark base, translucent white to cream-colored or light ochraceous buff, drying ochraceous buff to honey yellow, rarely zinc orange,[2] margin slightly

[1] Mycologist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

[2] Color readings made from Ridgway, R., Color standards and color nomenclature, Washington, D. C., 1912.

fimbriate; asci fusoid-clavate, slightly narrowed and rounded at the tip, gradually attenuated toward a short stipe, pore bluing with iodine, 8-spored, 25–40 × 4–6 μ, mostly about 35 × 5 μ; ascospores obliquely uniseriate to biseriate, hyaline, unicellular, clavate-ellipsoid or clavate-fusoid, guttulate, straight or slightly curved, 4.5–7 × 1.5–2.5 μ, mostly 5–6 × 2 μ; paraphyses filamentous, hyaline, longer than the asci, very slightly enlarged at the tips; exciple prosenchymatic, brown at the base, becoming hyaline at the margin, composed of narrow, thin-walled, shining hyphae; marginal hyphae hyaline, subclavate, faintly roughened and only slightly enlarged to 2–3 μ at the tips.

On bark of *Picea sitchensis*, Jan. 27–28, 1939, *H. E. Parks 6190* (TYPE); decorticated wood of *P. sitchensis*, Dec. 1948, *7255;* on old rust galls in bark of *Pinus muricata*, Mar. 1937, *5620* and Jan. 20, 1939, *6180;* on bark of *Tsuga heterophylla*, Feb. 4, 1939, *6210;* all collected by H. E. Parks, Spruce Cove, Trinidad, California.

The fungus is usually associated with resin in cracks of conifer bark, often on old rust galls; however, the specimen on decorticated wood of *Picea* (*7255*) does not appear to differ from the collections on bark and is therefore referred to the same species.

A *Cistella* on wood of *Sequoia* (*Parks 4224*) differs in having smaller asci and spores and sessile, hyaline apothecia, which lack the brown stem-like base of *C. parksii*. Several collections of another *Cistella* on *Juniperus* wood from Virginia were made by C. L. Shear. It would appear, therefore, that there are several species of the genus on coniferous wood in North America.

Dr. D. P. Rogers has pointed out the existence of *Cistella* Blume, a genus of the Orchidaceae antedating the fungus genus *Cistella* Quél. *Cistella* Blume (Bijdragen tot de Flora van Nederlandisch Indië, p. 293. 1825) is a synonym of *Geodorum* Jacks. (1810) and the former generic name is therefore not in use by phanerogamic botanists. *Cistella* Quél. (Quélet, Echiridion Fungorum p. 319. 1886), on the other hand, is an accepted genus of fungi emended in 1932 by Nannfeldt (Studien über die Morphologie und Systematik der nicht-lichenisierten operculaten Discomyceten, p. 265) and recognized by other mycologists. There appears to be no other existing generic name which can properly be applied to these discomycete species. In view of these facts, it is proposed that *Cistella* Quél. be conserved against *Cistella* Blume under the provision of Article 14 of the International Code of Botanical Nomenclature.

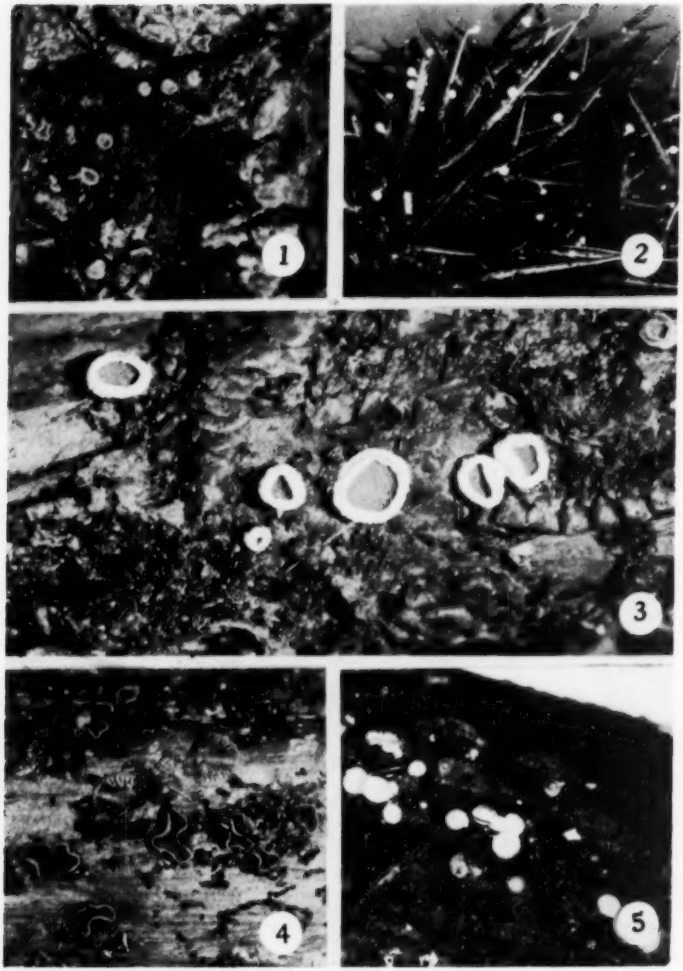

FIG. 1, *Cistella parksii* on *Pinus muricata*, × 10. FIG. 2, *Dasyscypha echinophila* on *Castanopsis sempervirens*, × 3.5. FIG. 3, *Haematomyxa tetraspora* on *Physocarpus capitatus*, × 10. FIG. 4, *Mollisia urticae* on *Urtica lyallii*, × 8. FIG. 5, *Mollisina ammophilae* on *Ammophila arenaria*, × 19. (Figs. 1, 2, 7, and 9 phot. R. L. Taylor; others phot. M. L. F. Foubert.)

2. **Colpoma californicum** sp. nov.

Apothecia in cortice immersa, cartilaginea, irregulariter elliptica vel subcircularia in ambitu, 2–3 mm in diametro, demum desciscentia, margine crasso contorto, atro, lacerato, hymenio melleo usque viridi-aureo, pruinoso; asci cylindrici, breve pedicellati, ad porum rotundatum attenuati, iodi ope non caerulescentes, octospori, 150–250 × 12–17 μ; ascosporae paralleles, aciculares, infra acutiores, hyalinae, multiseptatae, 150–175(–200) × 1.5–2 μ; paraphyses filiformes, hyalinae usque flavidulae, ad apices recurvatae, contortae et intertextae.

Apothecia immersed in the bark, cartilaginous, irregularly elliptical to subcircular in outline, 2–3 mm in diameter, margin thick, contorted, black, lacerate; apothecia eventually falling away leaving holes in the bark; hymenium honey-yellow to old gold, pruinose; asci cylindrical, short-pedicellate, narrowed at the apex to a small round pore, not bluing with iodine, 8-spored, 150–250 × 12–17 μ; ascospores parallel, acicular, slightly rounded at the tips and more acute below, hyaline, multiseptate, 150–175(–200) × 1.5–2 μ; paraphyses filiform, hyaline to pale yellowish, recurved, twisted and intertwined at the tips.

On bark of *Quercus chrysolepis,* Jonesville, Butte Co., May–June 1936, coll. *E. B. Copeland* (TYPE).

The apothecia of this species are shorter, rounder and more widely opened than those in *Colpoma quercinum* (Fr.) Wallr., from which it also differs in having much longer asci and spores.

3. **Dasyscypha echinophila** sp. nov. (FIG. 2)

Apothecia sparsa, superficialia, stipitata, molle ceracea, globosa dein cupulata, translucida nivea, tomentosa, 0.2–0.5 mm lata, 0.2–0.3 mm alta, stipite tomentoso, 0.2–0.3 × 0.1–0.2 mm; asci cylindrici, breve pedicellati, ad apices rotundati, iodi ope non caerulescentes, octospori, 28–33 × 2.5–3 μ; ascosporae oblique uniseriatae, aciculares usque fusoideae, unicellulares, hyalinae, 5–7 × 0.5–1 μ; paraphyses anguste lanceolatae, ascos superantes, tenui-tunicatae, 40–48 × 2–2.5 μ; excipulum hyalinum, nitidum, prosenchymaticum; pili hyalini, septati, ad apices asperati, 75–100 × 3–5 μ, saepe agglutinati et capitulum crystallinum 10–15 μ in diametro ferentes.

Apothecia scattered, superficial, stipitate, soft, waxy, at first spherical, then deep cup-shaped, pure white, translucent, tomentose, 0.2–0.5 mm broad, 0.2–0.3 mm deep, stipe tomentose, 0.2–0.3 × 0.1–0.2 mm; asci cylindrical, short-stipitate, rounded at the tips, not bluing with iodine, 8-spored, 28–33 × 2.5–3 μ; ascospores obliquely uniseriate, acicular-fusoid, unicellular, hyaline, 5–7 × 0.5–1 μ; paraphyses narrowly lanceolate, longer than the asci, 40–48 × 2–2.5 μ; excipulum hyaline, shining, prosenchymatous; excipular hairs hyaline, septate, roughened at the apex, 75–100 × 3–5 μ, often agglutinated and bearing globular masses of crystals 10–15 μ in diameter.

On involucre spines of *Castanopsis sempervirens,* Cow Creek, Stanislaus National Forest, Tuolumne Co., California, June 18, 1941, *C. R. Quick* (TYPE), ex herb. University of California.

Two species of Dasyscypha rather similar to this fungus in many respects, *D. nivea* (Hedw. ex Fr.) Sacc. and *D. virginea* (Batsch. ex Fr.) Fckl., both lack the masses of crystals on the excipular hairs. The hairs of *D. echinophila* resemble those of *D. crystallina* (Fckl.) Sacc., but the former differs in having much smaller asci and spores and shorter, narrower paraphyses.

4. **Haematomyxa tetraspora** sp. nov. (FIG. 3)

Apothecia e cortice emergentia, dispersa, sessilia, cupulata vel applanata, pallide grisea, molle carnosa, 0.5–1 mm in diametro, margine conspicuo, crasso, albo, pulverulento, hymenio vinaceo-griseo, sicco atro-ardosiaco, pruinoso, saepe excidente; asci longe stipitati, clavati, ad apices rotundati, poro ope iodi intense caerulescenti, tetraspori, 130–150 × 10–12 μ; ascosporae late ellipticae apicibus obtusis, uniseriatae, hyalinae, muriformes, 18–24 × 10–12 μ; paraphyses hyalinae, filiformes, dense ramosae et intertextae; excipulum dense plectenchymaticum, subhyalinum, hyphis superficialibus laxis ad marginem subfimbriatum protrudentibus praeditum.

Apothecia scattered, emerging from beneath the bark, sessile, shallow cup-shaped or flat, light neutral gray, soft fleshy, 0.5–1 mm in diameter, margin conspicuous, thick, white, pulverulent, hymenium purplish gray to vinaceous drab, drying dark neutral gray to blackish slate; hymenium often falling out and leaving empty cups when old; asci long stipitate, clavate, rounded at the apex, the pore staining intensely blue with iodine, 4-spored, 130–150 × 10–12 μ; ascospores uniseriate in the upper end of the ascus, broadly elliptical, hyaline, muriform, with 2 to 5 horizontal and 1 or 2 longitudinal septa, 18–24 × 10–12 μ; paraphyses hyaline, filiform, densely branched and interwoven; exciple densely plectenchymatic, hyaline or slightly greenish, the outer layer of hyphae with loose, projecting ends forming a slightly fimbriate margin.

On twigs of *Physocarpus capitatus,* Darlingtonia, Del Norte Co., California, Apr. 1940, *H. E. Parks 6438* (TYPE).

Microscopic mounts were made from the type specimen of *Haematomyxa ascoboloides* Ell. & Ev., which is close to the Californian fungus in the dimensions of spores and asci. In *H. ascoboloides* the ascospores are narrower, more strongly narrowed at the ends, and with fewer septa. The apothecia are also much smaller, with dark margins, and the asci are 8-spored. *H. sequoiae* (Plowr.) Bonar, described from California, has much larger asci and spores, as is the case in *H. rufa* (Ell. & Ev.) Rehm and *H. pakistani* Müller & Ahmad.

5. **Helotium polystichi** sp. nov.

Apothecia sparsa, longe stipitata, carnoso-ceracea, patellata dein convexa, 0.5–1.5 mm in diametro, pallide flavidula vel ochracea, sicca cinnamomea, hymenio concolori, margine glabra, stipite 0.5–1.5 × 0.2–0.5 mm; asci cylindrici, basim versus gradatim attenuati, ad apicem abrupte angustati, obtusi et crasse tunicati, iodi ope caerulescentes, octospori, 140–150 × 11–13 μ; ascosporae biseriatae, hyalinae, fusoideae vel fusoideo-clavatae, infra vel utrinque acutae, unicellulares, rectae vel saepius curvatae, guttulatae, non ciliatae, 35–45 × 4–5 μ; paraphyses numerosae, hyalinae vel flavidulae, eramosae, ad apices leniter usque ad 1.5 μ incrassatae, agglutinatae; excipulum prosenchymaticum, subhyalinum vel pallide brunneum.

Apothecia scattered, long-stipitate, fleshy-waxy, patellate to convex, 0.5–1.5 mm in diameter, cream-buff or light ochraceous to yellowish ocher, drying pinkish cinnamon, hymenium concolorous, pruinose, margin even, stipe smooth, 0.5–1.5 × 0.2–0.5 mm; asci cylindrical, gradually narrowed below, rather abruptly narrowed toward the flattened, thick-walled apex, pore bluing with iodine, 8-spored, 140–150 × 11–13 μ; ascospores biseriate, hyaline, fusoid to clavate, acute at both ends or more so below, unicellular, biseriate, straight or more often curved, often with one large central guttulte, or sometimes with several smaller oil drops, not ciliate, 35–45 × 4–5 μ; paraphyses numerous, hyaline to pale yellowish, flexuous, filiform, unbranched, slightly thickened up to 1.5 μ at the tips, agglutinated into a yellowish mazaedium; exciple prosenchymatous, subhyaline to pale brownish.

On stems of *Polystichum munitum*, Spruce Cove, Trinidad, California, Jan. 16, 1941, *H. E. Parks 6476* (TYPE).

This *Helotium* differs from *H. ceipi* var. *struthiopteridis* Velenovsky, described on *Matteuccia* (*Struthiopteris*) from Bohemia, in having less acute spores. The extremely long spores distinguish it from other large-spored species of the genus found on herbaceous stems such as *H. scutula* (Pers. ex Fr.) Karst., to which it bears a superficial resemblance.

6. **Mollisia lithocarpi** sp. nov.

Apothecia amphigena, sessilia, sparsa, brunnea, molle carnosa, subglobosa dein cupulata, 0.2–0.5 mm in diametro, margine subfimbriato-dentato, sicco involuto, hymenio pallide fumoso; asci anguste cylindrici, basim versus gradatim attenuati, ad apices subangustati, poro iodi ope caerulescente, octospori, 33–35 × 3–3.5 μ; ascosporae oblique uniseriatae, hyalinae, elliptico-fusoideae, unicellulares, 4–5 × 0.7–1 μ; paraphyses filamentosae, hyalinae, eramosae, ascos superantes; textura excipuli pseudoparenchymatica, ex cellulis brunneis crasse tunicatis, 10–12 μ in diametro, marginem versus clavatis (20–30 × 4–5 μ) et caespitosis composita.

Apothecia amphigenous, mostly hypophyllous, sessile, scattered, soft fleshy, subglobose to shallow cup-shaped, 0.2–0.5 mm in diameter, olive

brown to fuscous brown, margin fimbriate, dentate, inrolled when dry, hymenium pale smoke gray; asci narrow cylindrical, gradually narrowed toward the base, rounded and very slightly attenuated at the apex, pore blue with iodine, 8-spored, 33–35 × 3–3.5 μ; ascospores obliquely uniseriate, hyaline, unicellular, elliptical-fusoid, 4–5 × 0.7–1 μ; exciple pseudoparenchymatous, composed of round, brown thick-walled cells 10–12 μ in diameter, becoming clavate (20–30 × 4–5 μ) and united in irregular tufts at the margin.

On dead leaves of *Lithocarpus densiflora*, California: Smith River, Del Norte Co., Apr. 1933, *H. E. Parks 4222;* Orick-Orleans Grade, June 1935, *Parks 5557;* Big Basin State Park, Santa Cruz Co., July 15, 1954, *L. Bonar* (TYPE).

Mollisia prinicola (Ell.) Sacc., found on leaves of *Quercus prinos*, has broader asci and longer spores. *M. lithocarpi* frequently occurs in leaf spots caused by various other fungi with which it is associated.

7. **Mollisia urticae** sp. nov. (FIG. 4)

Apothecia sessilia, patelliformia, molle carnosa, ardosiaca usque ardosiaco-atra, 0.8–1.2 mm in diametro, hymenio pallide ardosiaco-griseo, margine angusto, pallido; asci anguste cylindrici vel cylindrico-clavati, longe stipitati, iodi ope non caerulescentes, octospori, 55–65 × 5–8 μ; ascosporae 1–2-seriatae, hyalinae, unicellulares, cylindrico-fusoideae usque cylindrico-clavatae, 9–10 × 1.5–2 μ; paraphyses hyalinae, filiformes, non incrassatae; excipulum pseudoparenchymaticum, e cellulis atro-brunneis tenuibus hexagonis 15–20 μ in diametro compositum, ad marginem hyphis hyalinis tenuibus conglutinatis et ad basim hyphis raris brunneis praeditum.

Apothecia sessile, shallow cup-shaped to patellate, soft fleshy, slate gray to slate black, smooth, 0.8–1.2 mm in diameter, hymenium pale neutral gray to slate gray, margin narrow, paler and elevated, contorted and wavy on drying; asci narrow cylindrical to cylindrical-clavate, long stipitate, narrowed at the apex, not bluing with iodine, 8-spored, 55–65 × 5–8 μ; ascospores uniseriate below, biseriate above, hyaline, guttulate, unicellular, cylindrical-fusoid to narrow clavate, 9–10 × 1.5–2 μ; exciple pseudoparenchymatic, composed of dark-brown, thin-walled hexagonal cells 15–20 μ in diameter, smaller toward the margin and changing to narrow, hyaline, conglutinate hyphae; brown septate hyphae rarely present near the base of the apothecium.

At the base of living stems of *Urtica lyallii*, McNeil's Creek and Hobson Creek, Trinidad, California, Feb. 1941, *H. E. Parks 6601* and *6602* (TYPE).

One hesitates to add to the bewildering number of species of *Mollisia* already described on herbaceous stems, but this fungus does not appear to agree with any descriptions or specimens available. *Mollisia urtici-*

cola Phill., described on *Urtica*, differs in color and in having smaller spores. The California species is somewhat similar in macroscopic appearance to *M. cinerea* (Batsch. ex Fr.) Karst., but its occurrence on living stems makes it unlikely that it could be specifically identical with that common lignicolous species, from which it is also distinct in having narrower, more pointed spores. It also resembles *M. adenostylidis* Rehm, but shows no trace of the globular or pear-shaped projecting cells reported for Rehm's species by Gremmen (**4**, p. 8–9, pl. 2, F).

8. **Mollisina ammophilae** sp. nov. (FIG. 5)

Apothecia superficialia, conferta vel sparsa, ceraceo-carnosa, obconica, supra applanata, 0.1–0.2 mm in diametro, cremea, hymenio concolori, margine aequali; asci cylindrico-clavati, ad apices rotundati, basim versus gradatim attenuati, poro iodi ope caerulescente, octospori, 24.5–26.4 × 3.5–4 μ; ascosporae biseriatae, hyalinae, unicellulares, aciculares, utrinque acutae, rectae vel subcurvatae, 4.5–5 × 0.7–1 μ; paraphyses filiformes, eramosae, hyalinae; excipulum subhyalinum, pseudoparenchymaticum, e cellulis rotundis tenui-tunicatis, marginem versus minoribus compositum; hyphae marginales rarae, tenues, filiformes, glabrae, 1–1.5 μ in diametro, interdum in caespitibus conglutinatae.

Apothecia superficial, crowded or scattered, sessile, waxy-fleshy, obconic, flattened above, exterior and hymenium cream-colored, 0.1–0.2 mm in diameter, margin uneven but not dentate; asci cylindrical-clavate, rounded at the tips, gradually narrowed toward the base, pore bluing with iodine, 8-spored, 24.5–26.4 × 3.5–4 μ; ascospores biseriate, hyaline, unicellular, acicular, acute at both ends, straight or slightly curved, 4–5.5 × 0.7–1 μ; paraphyses filiform, unbranched, hyaline; exciple subhyaline, pseudoparenchymatous, composed of round, thin-walled cells, much smaller at the margin; marginal hyphae scarce, thin, filiform, smooth, 1–1.5 μ in diameter, sometimes agglutinated in tufts.

On stems of *Ammophila arenaria*, Little River sand dunes, Humboldt Co., California, Jan. 2, 1940, *H. E. Parks 6416* (TYPE).

This very inconspicuous fungus is scarcely distinguishable, even when moist, from the minute whitish grains of sand commonly present on the stems of the host, and is nearly invisible when dry. In Dennis's key to the Hyaloscyphaceae (**3**, pp. 1–2) it comes closest to *Mollisina* Hoehn. (**6**, p. 67), its essential features agreeing with the characters of this genus as described and illustrated by Dennis (**3**, p. 85). The excipular hairs are scanty and inconspicuous, not acute as in *Hyaloscypha* or rounded and asperate as are those in *Cistella*. Nor do they form definite teeth as in *Pezizellaster*. The very small, round cells at the margin of the apothecia are a noticeable features of the genus. The two previously described species occur on rotting leaves in Europe.

9. **Pezizellaster herbarum** sp. nov. (FIG. 6)

Apothecia superficialia, substipitata vel ad basim obconicum contracta, dense aggregata, globosa dein cupulata vel contorta, 0.2–1 mm in diametro, molle carnosa, roseo-bubalina vel pallide cinnamomea, albo-pruinosa, margine dentato, pallidiore, hymenio ochraceo-salmoneo; asci cylindrico-clavati, ad apices attenuati, iodi ope non caerulescentes, octospori, 50–65 × 4–8 μ; ascosporae irregulariter biseriatae, cylindraceo-clavatae usque anguste fusoideae, hyalinae, unicellulares, guttulatae, rectae vel subcurvatae, 8–12(–15) × 1.5–2 μ; paraphyses filiformes, hyalinae, ad apices usque 1.5–2 μ inflatae, ascos superantes; excipulum subhyalinum, prosenchymaticum; hyphae marginales hyalinae vel flavidulae, perangustae, obtusae, septatae, interdum recurvatae, interetextae et in dentes triangulares agglutinatae.

Apothecia superficial, densely aggregated, substipitate or narrowed below to an obconic base, at first globose, then cupulate, often contorted by mutual pressure, 0.2–1 mm in diameter, soft-fleshy, pinkish-buff to cinnamon buff, white pruinose; hymenium pale pinkish-buff, drying vinaceous cinnamon to light ochraceous salmon, margin dentate; asci cylindrical-clavate, narrowed at the apex, not bluing with iodine, 8-spored, 50–65 × 4–8 μ; ascospores irregularly biseriate, cylindrical-clavate to narrow fusoid, hyaline, one-celled, guttulate, straight or slightly curved, 8–12(–15) × 1.5–2 μ; paraphyses filiform, hyaline, slightly inflated to 1.5–2 μ at the tips, outranking the asci; exciple subhyaline, prosenchymatic; marginal hyphae hyaline to pale yellowish, septate, obtuse, sometimes recurved, twisted and tangled, often agglutinated into triangular pointed fascicles forming the dentate margin.

On dead stems of *Scrophularia californica*, McNeil's Creek, Trinidad, California, Feb. 9, 1941, *H. E. Parks 6588;* of *Dahlia* sp., Spruce Cove, Trinidad, Nov. 1948; and of *Urtica lyallii*, associated with *Leptosphaeria* sp., Hobson Creek, Trinidad, Feb. 1, 1941, *H. E. Parks 6603* (TYPE).

It might be possible to consider placing this discomycete in the genus *Dasyscypha*. The fasciculate hyphae at the margin of the apothecia are, however, scarcely distinct enough to be considered as hairs characteristic of that genus, and the greater part of the apothecium is entirely smooth. The elongated hyphae, agglutinated in groups and forming a dentate margin, agree with those in *Pezizellaster* Hoehn. as described by the author of that genus (**5**, p. 349) and by Dennis (**3**, p. 61). The apothecia of *Pezizellaster herbarum* are larger and the asci and spores longer than those of *P. confusus* Hoehn. and *P. tami* (Lamy) Hoehn., described on herbaceous stems from Europe.

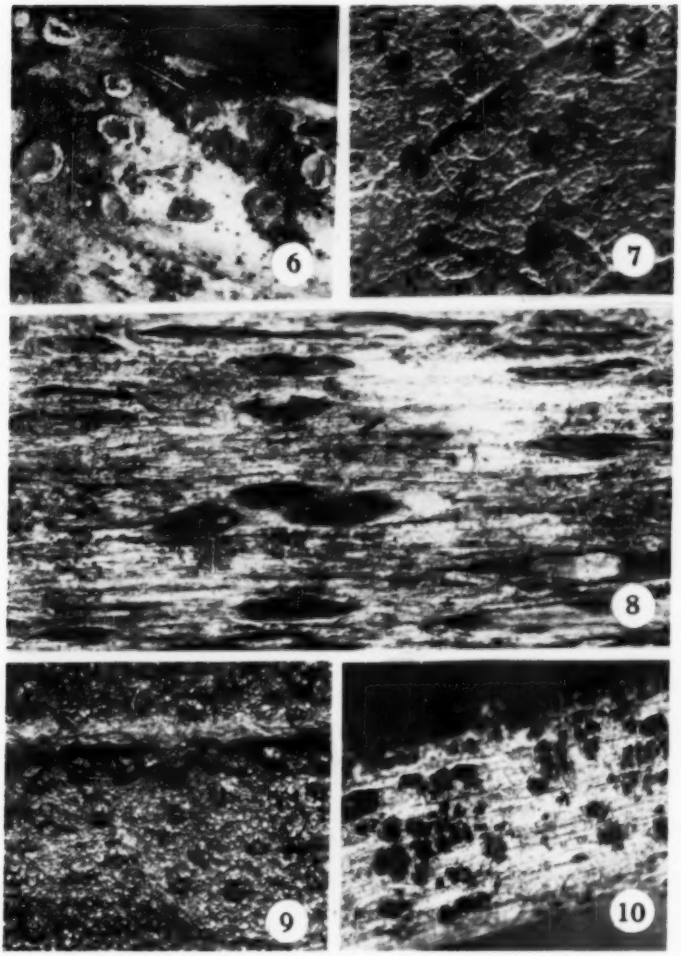

FIG. 6, *Pezizellaster herbarum* on *Urtica lyallii*, ×12. FIG. 7, *Phacociboria garryae* on *Garrya elliptica*, ×4. FIG. 8, *Propolidium californicum* on *Salvia leucophylla*, ×10. FIG. 9, *Stictis myricae* on *Myrica californica*, ×8. FIG. 10, *Tryblidaria californica* on composite, ×8. (Figs. 1, 2, 7, and 9 phot. R. L. Taylor; others phot. M. L. F. Foubert.)

10. **Phaeociboria garryae** sp. nov. (FIG. 7)

Apothecia plerumque hypophylla, rarius epiphylla, conspersa, turbinata vel substipitata, cartilaginosa, 1–2 mm in diametro, olivaceo-brunnea, sicca fere nigra, hymenio concolori, margine glabro, leniter involuto, stipite 0.2–0.5 mm alto, 0.5 mm lato; asci cylindrici, ad apices obtusati, gradatim basim versus attenuati, iodi ope non caerulescentes, 4–8-spori, 120–150 × 11–13 μ; ascosporae uniseriatae, brunneae, unicellulares, late ellipsoideae usque late fusoideae, inaequilaterales, 2- usque multiguttulatae, verrucosae, 15–20 × 8–12 μ; paraphyses filiformes, hyalinae, ad apices usque ad 3 μ inflatae et subgranulosae; excipulum e strato hypotheciali pallide brunneo, strato medullari crasso, hyalino, laxe intertexto, et cortice pallidebrunneo, subfurfuraceo compositum.

Apothecia usually hypophyllous, rarely epiphyllous, scattered, turbinate to substipitate, cartilaginous, 1–2 mm in diameter, Verona brown to olive brown, hymenium concolorous, drying wood brown to Natal brown, with hymenium olive brown to olivaceous black, margin smooth, stipe 0.2–0.5 × 0.5 mm; asci cylindrical, obtuse at the tips, gradually narrowed toward the base, not bluing with iodine, 4–8-spored, 120–150 × 11–13 μ; ascospores obliquely uniseriate, pale brown to deep golden brown, unicellular, broad-ellipsoid to broad-fusoid, with two to many guttules, verrucose, with small warts arranged in lines, 15–20 × 8–12 μ, often only 4 spores maturing; paraphyses filiform, hyaline, unbranched, slightly inflated and subgranulose at the tips; excipulum of several layers; hypothecium thin, pale brown, medullary tissue thick, made up of hyaline shining, loosely interwoven hyphae, 5–7 μ in diameter; cortex of pale brownish parallel hyphae, the loose ends making the cortex slightly furfuraceous.

On dry leaves of *Garrya elliptica* on cut brush, Spruce Cove, Trinidad, California, Jan. 1, 23, and 27, 1947, *H. E. Parks 6943, 6999,* and *7008* (TYPE).

Mr. Parks noted that the affected leaves were all collected from dead brush cut the previous summer and from which the leaves had not yet fallen off. The fungus was not present on naturally dead, fallen leaves.

In the structure of the apothecium and other essential characters the fungus agrees with description of the genus *Phaeociboria* Hoehn. as emended by Whetzel and White (**7**, p. 614). The ascospores are broader than those of *P. tetrica* (Quél.) Hoehn. and become verrucose at maturity.

11. **Phialea umbellulariae** sp. nov.

Apothecia hypophylla, sparsa, stipitata, molle carnosa, pellucida, cupulata dein patellata usque convexa, griseo-flavidula vel avellanea, sicca obscurioria usque subatra, 0.5–0.8(–1) mm in diametro, margine laevi vel minute crenato, stipite 0.5–1 × 0.2 mm, hymenio pallide griseo; asci longe stipitati, anguste cylindrici, apices

versus attenuati, octospori, iodi ope non caerulescentes, 40–45 × 3–3.5 μ; ascosporae biseriatae, hyalinae, aciculari-clavatae, guttulatae, 3.5–4 × 0.5–1.5 μ; paraphyses filiformes, hyalinae usque pallide flavidulae, ad apices subinflatae et granulosae; excipulum prosenchymaticum, hyalinum, extus hyphis tenuitunicatis, marginem versus tenuioribus, densioribus et parallelis praeditum.

Apothecia hypophyllous, sparse, stipitate, soft fleshy, translucent, cup-shaped becoming patellate to convex, light drab or vinaceous buff to avellaneous, darker to nearly black when dry, minutely furfuraceous, 0.5–1 mm in diameter, margin even, thin, finely crenate, inrolled when dry, stipe 0.5–1 × 0.2 mm, ochraceous tawny, hymenium pale smoke gray to drab gray; asci long stipitate, narrow cylindrical, rounded at the tips, not bluing with iodine, 8-spored, 40–45 × 3–3.5 μ; ascospores biseriate, hyaline, acicular-clavate, guttulate, 3.5–4.5 × 0.5–1.5 μ; paraphyses filiform, unbranched, hyaline to pale yellowish, slightly swollen and granulose at the tips; exciple prosenchymatic, hyaline, the outer layer of subhyaline, thin-walled, large-celled hyphae which break up and give the apothecium a furfuraceous appearance, hyphae thinner, more densely compact and parallel toward the margin.

On dead leaves of *Umbellularia californica*, Darlingtonia, Del Norte Co., California, Jan. 1938, *H. E. Parks 6093* (TYPE), Jan. 1939, *6374*, and Jan. 19, 1947, *6982*, associated with *Ceuthospora* sp. and various other fungi.

Phialea umbellulariae is a very inconspicuous species, nearly invisible when dry, particularly when other more striking fungi are present. Among similar small-spored species of *Phialea* on leaves, *P. pertenuis* Sacc. differs from this fungus in having ovoid spores. *Helotium cecropiae* P. Henn. on leaves of *Cecropia* from Brazil may be distinguished from it by its color. The apothecia, asci and spores are larger in *H. caracassensis* Dennis on dead leaves in Venezuela.

12. **Propolidium californicum** sp. nov. (FIG. 8)

Apothecia ligno dealbato immersa, sparsa, elongato-elliptica usque fusoidea et utrinque acuta, ligno atrato subelevato circumcincta, 1–2 × 0.5 mm vel usque 4 mm confluentia, hymenio viridi, sicco subatro; asci late cylindrico-clavati, ad apices obtuse rotundati, abrupte in pedicellum brevem attenuati, octospori, iodi ope non caerulescentes, 45–55 × 6–8 μ; ascosporae 1–2-seriatae, hyalinae, cylindricae, utrinque rotundatae, 1–3-septatae, rectae vel curvatae, 11–15 × 2–3.5 μ; paraphyses numerosae, hyalinae vel pallide virescentes, ad apices ramosae et subinflatae, recurvatae et in epithecium intertextae; hypothecium tenue subhyalinum vel viridulum, prosenchymaticum.

Apothecia scattered, occasionally confluent, immersed in whitened areas of wood, elongate-elliptical to fusoid, sharply acute at the ends, the elevated host tissue forming a dark margin, 1–2 × 0.5 mm or up to

4 mm long when confluent; hymenium dark ivy green to nearly black; asci broadly cylindrical-clavate, obtusely rounded at the tips, abruptly narrowed to a short pedicel, not turning blue with iodine, 8-spored, 45–55 × 6–8 μ; ascospores 1–2-seriate, hyaline, cylindrical with rounded ends, 1–3-septate, straight or curved, 11–15 × 2–3.5 μ; paraphyses numerous, hyaline to greenish, branched, slightly inflated and recurved at the tips and interwoven to form a mazaedium; hypothecium thin, subhyaline to greenish, prosenchymatous.

On wood of *Salvia leucophylla* (*Audibertia nivea*), Elsinore, California, Apr. 30, 1939, *C. L. Shear* (TYPE).

The blue-green hymenium of this fungus is suggestive of many species of *Durella*, but the apothecia remain immersed in the wood of the host, not becoming superficial as in the latter genus. The raised border is formed of darkly stained wood fibers and not composed of fungus hyphae. Neither this nor the following species, also described as *Propolidium*, shows any bluing reaction with iodine and the fungus therefore cannot be placed in *Cryptodiscus*. *Xylogramma nigerrima* (Ell. & Ev.) Rehm, a Californian species occurring on *Rhus*, differs in having broader, subglobose asci, twice the width of those in *Propolidium californicum*.

13. **Propolidium shearii** sp. nov.

Apothecia plana, in ligno immersa, circularia, ellipsoidea vel irregularia in ambitu, 1-1.5 × 1 mm, interdum confluentia usque 2-3 mm, extremis rotundis vel acutis, hymenio ardosiaco, sicco atro-ardosiaco vel atro-viridi, margine albido e ligno composito; asci cylindrici, iodi ope non caerulescentes, ad bases attenuati, ad apices rotundati et tunica valde incrassata, 70–90 × 15–18 μ; ascosporae 2-3-seriatae, cylindricae, subhyalinae, rectae vel curvatae, 3-7-septatae, 25–33 × 5–6 μ; paraphyses filiformes, ad apices inflatae et obscuriores, mazaedium caeruleo-viridem efformantes.

Apothecia immersed in wood, flat, 1–1.5 × 1 mm, circular, elliptical or irregular in outline, hymenium slate gray, drying blackish slate, with whitish margin formed by the host tissue; asci cylindrical, narrowed toward the base, rounded at the apex with wall much thickened, not bluing with iodine, 70–90 × 15–18 μ; ascospores 2–3-seriate, cylindrical, hyaline, straight or curved, 3–7-septate, 25–33 × 5–6 μ; paraphyses filiform, thickened and darker at the tips, forming a blue-green mazaedium.

On decorticated wood of *Quercus*, Ortego, California, Apr. 20, 1939, *C. L. Shear* (TYPE).

Feltgen described three species of this genus, *Propolidium pallescens*, *P. acerinum* and *P. rehmianum*, all with spores measuring more than 30 μ in length; these species differ, however, from *P. shearii* in the shorter asci and the color of the hymenium.

14. **Stictis myricae** sp. nov. (FIG. 9)

Apothecia hypophylla, in maculis circularibus vel irregularibus dense aggregata vel per superficiem folii totam dispersa, 0.3–0.5 mm in diametro, 3–6-lobata, hymenio vitreo-viridi, pruinoso; asci cylindrici, breviter pedicellati, ad apices subacuminati, octospori, poro iodi ope caerulescenti, 80–115 × 6–7 μ; ascosporae aciculares, hyalinae vel viridulae, paralleles, rectae, multinucleatae usque 3–5-septatae, 60–80 × 1–1.5 μ; paraphyses hyalinae, apices versus ramosae.

Apothecia hypophyllous, densely aggregated in circular to irregular spots 3–5 mm in diameter or scattered over nearly the entire lower leaf surface, subcircular to angular, 0.3–0.5 mm in diameter, opening by 3 to 6 lobes, hymenium sea-foam green, pruinose; asci cylindrical, short pedicellate, subacuminate at the apex, pore bluing with iodine, 8-spored, 80–115 × 6–7 μ; ascospores acicular, hyaline to greenish, parallel, straight, multinucleate to 3–5-septate, 60–80 × 1–1.5 μ; paraphyses hyaline, branched near the tips.

On leaves of *Myrica californica*, Spruce Cove, Trinidad, California, Jan. 24, 1947, *H. E. Parks 7005* (TYPE).

Stictis myricae is very close to *S. vaccinii* Ell. & Ev. both in general appearance and in microscopic characters, so that is named as a new species with some hesitation. The hosts of the two fungi are not related, belonging to different families. The asci and spores of *S. myricae* are longer than those of the fungus on *Vaccinium* and the spores are parallel and straight, not spirally coiled in the asci, as is the case in the latter. The fungus on *Myrica* is therefore described as a distinct species.

15. **Tryblidaria californica** sp. nov. (FIG. 10)

Apothecia sessilia, 0.2–0.5 mm in diametro, in caulibus dealbatis dispersa, coriacea, depresso-globosa dein cupulata usque patellata, fuligineo-atra usque atro-purpurea, hymenio concolori vel pallidiore, margine conspicuo, lato; asci cylindrico-clavati, breve pedicellati, ad apices obtusi, iodi ope non caerulescentes, octospori, 100–110 × 15–20 μ; ascosporae irregulariter uniseriatae, oblongo-ellipsoideae, utrinque obtusae, flavidulae usque olivaceo-brunneae, muriformes, horizontaliter 3-septatae, ad septum medium leniter constrictae, cellulis centralibus longitudinaliter septatis, 20–24 × 9–13 μ; paraphyses filiformes, ad apices inflatae et brunneae, in mazaedium crassum atrum agglutinatae; hypothecium crassum, hyalinum; cortex brunneus, pseudoparenchymaticus.

Apothecia sessile, 0.2–0.5 mm in diameter, scattered on whitened stems, coriaceous, depressed globose then deep cupulate to patellate, sooty black to dull purplish black, hymenium concolorous or slightly paler, margin thick, dark, conspicuous; asci cylindrical-clavate, short pedicellate, obtuse at the tips, not bluing with iodine, 8-spored, 100–110 × 15–20 μ; ascospores irregularly uniseriate, oblong-ellipsoid, obtuse at

both ends, yellowish to olivaceous brown, muriform, with 3 horizontal septa, slightly constricted at the central septum, the two center cells longitudinally septate, 20–24 × 9–13 μ; paraphyses filiform, swollen and brown at the tips, anastomosing to form a thick dark mazaedium; hypothecium thick, hyaline; cortex pseudoparenchymatous, brown.

On stems of a composite, Parris, California, Mar. 11, 1939, *C. L. Shear* (TYPE).

Most species of *Tryblidaria* have larger spores than those of *T. californica*, while those agreeing in spore measurements differ in spore septation and other characters.

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THE FIRST RECORDED TRUFFLE FROM TEXAS [1]

CHARLES HEIMSCH

(WITH 5 FIGURES)

Tuber texense sp. nov.

Ascoma 2.0–3.5 cm diametro brunneum scabrum vel minute impariterque verrucosum regulare vel sublobatum cum una vel sulcis pluribus profundis lunaribus. Gleba marmorata. Cortex exterior pseudoparenchymaticus tunc dense prosenchymaticus demum in hyphas steriles densas immutans quae in gleba venas internas irregulariter dilatatas vel angustatas formant; venae externae paucae plerumque magnae laxae secus sulcas aperientes. Asci stipitati subglobosi vel elongati 1–5- (plerumque 4-) spori; venter 36–47 × 58–68 μ; stipes 15–25 μ; sporae breviter vel longe ellipsoideae 14–26 × 22–40 μ flavescentes spinosae vel plus minusve spinosuloreticulatae spinis 2 μ longis.

Ascocarp 2–3.5 cm in diam., light brown, scabrous to minutely and irregularly verrucose, regular to somewhat lobed, exhibiting one to several deep crescentic to irregular furrows; gleba conspicuously marbled; outer cortical layer pseudoparenchymatous, changing gradually to compact prosenchyma and finally to compact masses and strands of sterile hyphae, these continuing into the gleba to form the irregularly widened and narrowed venae internae; the venae externae few, mostly large, loose-textured, opening to the surface at the furrows; asci stipitate, subglobose to elongate, 1–5- (generally 4-) spored, the venter 36–47 × 58–68 μ, the stipe 15–25 μ; spores short- to long-ellipsoid, 14–26 × 22–40 μ, yellowish, spinose to more or less spinose-reticulate, the spines 2 μ long.

Texas: Austin, *Charles Heimsch 1*, TYPE (TEX).

Knowledge of the hypogeous ascomycetes or Tuberales in America has been developed only within the last 70 years. The early contributions of this period were largely concerned with collections from the state of California, and these were the subject of extensive studies by Harkness (**1899**) and Gilkey (**1916**). A subsequent study included collections from nineteen states, the District of Columbia, and four provinces of Canada (Gilkey, **1939**). In the latest extensive treatment of

[1] The writer expresses appreciation to Dr. Helen M. Gilkey for advice and preparation of the technical description and to Dr. Lloyd H. Shinners for preparation of the Latin diagnosis.

the group (Gilkey, **1954**), species are cited as distributed in twenty-three states, the District of Columbia, four provinces of Canada and Alaska. As far as is known, the collection reported here constitutes the first record of these fungi from the state of Texas.

The fruiting bodies which are the subject of the present report were discovered in Austin while cultivating the soil in a planting bed at the base of a pecan tree. The soil had been removed to a depth of from six to eight inches, and while it was being replaced the fungus structures were noted. Any attachments the ascocarps may have had were obliterated with disturbance of the soil, but on the basis of their location when noted, it may be assumed that they were originally in fairly close proximity.

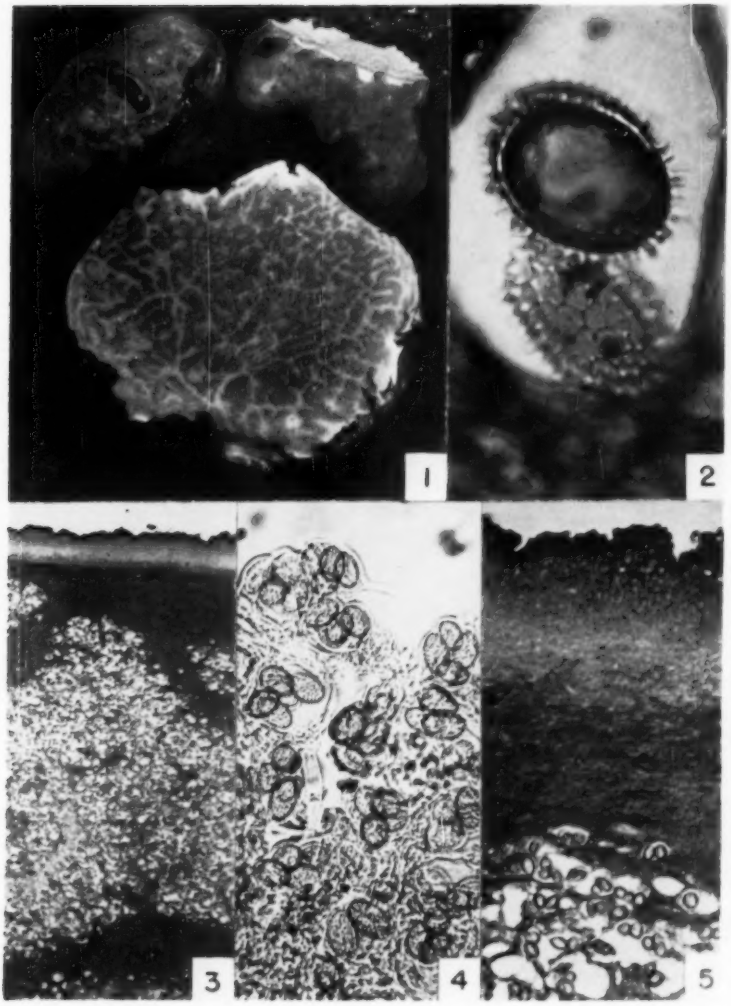
Fine roots of the tree ramified extensively throughout the soil that was removed. Although an association between the fungus and the roots might have existed, evidence for it was lacking or had been destroyed. Regardless of such conjecture, this finding represents what is believed to be the first recorded instance of any ascocarps of the Tuberales being found in the proximity of or in association with pecan.

Description of ascocarps: The collection consists of three ascocarps, two of which were damaged by the spade (FIG. 1). The largest is at least 3.5 cm in diameter, and the largest dimension of the others is about 2.5 cm. In shape, the largest is approximately subspherical, whereas the smaller specimens may be regarded as irregular cylinders about twice as long as wide. Their color, as recalled, was light brown when found.

Ascocarp surfaces are relatively smooth with conspicuous crescentic to irregular deep furrows as seen on the bottom left and middle of the specimen (FIG. 1, upper left). At one place on the cut surface of the largest specimen a vein terminates at such a groove, although this is not apparent in the photograph. In the intact specimen, more extensive and rougher surface markings can be noted at the right. Almost one half of the outer surface of the largest specimen (underside, as photographed) has such a marking. These areas may have arisen from splitting of the cortex during diameter growth and there followed some proliferation of internal hyphae thus exposed.

A portion of the large ascocarp was removed and prepared for sectioning by embedding in paraffin; sections were stained with Haidenhain's haematoxylin and safranin. Cortex thickness in the sectioned portion was measured approximately as 175–225 μ for the outer layer and 225–260 μ for the inner layer (FIGS. 3, 5).

Some of the variation in spore size and number per ascus can be noted in FIG. 4. The reticulate nature of the spore surface (FIG. 2)



FIGS. 1–5. *Tuber texense*. 1. Ascocarps showing surface features and marbled gleba, × 1.5. 2. Spores in microtome section showing spinose-reticulate character in equatorial and surface views, × 970. 3. Microtome section of ascocarp showing relationship of cortex to venae internae, × 19. 4. Razor blade section (unstained) showing spores of varying number and size in different asci, × 170. 5. Cortex detail; compare with FIG. 3 and text, × 125.

was detected in the sectioned material, the characteristic being virtually indiscernible in intact spores. In the outermost gleba of the sectioned material spore-surface reticulation is not prominent and the spines are generally shorter than those of spores in the innermost gleba. Most of these latter that have the wall in proper section show some evidence of reticulation, but commonly the pattern is not so well developed nor as regular as that figured. When reticulation is present most spines are included in the pattern, although the occurrence of solitary spines in the alveoli is not uncommon, particularly in areas where spines are most widely spaced.

Systematic considerations: Among the species of *Tuber*, spiny spores are not common, this characteristic having been indicated, in American species, for only two, *T. candidum* Hark. and *T. Harknessii* Gilkey (Gilkey, **1954**), but ascocarps of the latter bear small resemblance to those here described. In neither of these species, however, is the spinose condition of the spores seen to be associated with a surface reticulum. This fact, in association with differences in spore size and shape and shape of the ascus, serves to distinguish the ascocarps under consideration from those of *T. candidum*. It is concluded, therefore, that these fruiting structures represent an hitherto undescribed species of *Tuber*.

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ADDITIONAL SPECIES OF OPHIOSTOMATACEAE FROM COLORADO

Ross W. Davidson [1]

The earlier investigations of sapwood-staining fungi were conducted mainly in the eastern states (**1**, **2**, **3**, **6**, and **8**). Rumbold (**8**, **9**, and **10**) described a few associated with bark beetles in the west, but these papers do not represent comprehensive studies of wood-staining fungi in the western states.

In more recent years Davidson (**4** and **5**) has reported a few species, mostly Ophiostomataceae. These studies represent only fragmentary information gathered while conducting other forest disease research.

During the past two years additional information on species of the Ophiostomataceae has been obtained. Such information indicates the need for more extensive and intensive studies of this group of fungi from the standpoint of their distribution, host relationship, and insect associations. However, for the present, only descriptions of the fungi can be given, with incomplete information on their biological relationships. Some cause staining of bark and sapwood of host trees; others probably cause very little discoloration of the substratum.

A recent monographic study by Hunt (**7**) places all species in a single genus, *Ceratocystis*. This avoids the difficulty of generic classification with certain species intermediate between natural groups—as *Ophiostoma* and *Grosmannia*. However, the conidial stages seem more useful than the perithecia in indicating natural relationships.

SPECIES ISOLATED FROM SUBALPINE FIR

Preliminary examination of dead or dying subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) in several localities occasionally revealed darkly stained canker-like areas. Some contained insect galleries; others did not. A bark insect common to such trees is *Dryocetes confusus* Sw., but other bark insects, either primary or secondary, may also have been

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present. Although isolations have been made from perithecia fruiting in such insect galleries, the writer has no detailed data on the insect-fungus relationship.

One of the staining fungi isolated is a species of the genus *Leptographium*. Since no perithecial stage was definitely shown to be connected with it, a description will not be given here.

CERATOCYSTIS PENICILLATA (Grosm.) Moreau

Perithecia in insect galleries and occasionally developing in cultures, black, superficial, or with base imbedded, large globose, smooth, 180–230 μ in diameter; necks black, long, 650–1500 μ, 45–60 μ diameter at base to 20–25 μ at tip; no ostiolar hyphae seen; ascospores curved, similar to segment of an orange, hyaline, 3.8–5.5 × 1.4–2.0 μ.

Cultures hyaline at first, later with conspicuous coarse light gray to black mycelium; perithecia developing in older cultures in 3 to 5 weeks on surface of substratum, with necks protruding through the mat of dark gray surface mycelium; growth medium to fast, 35 mm;[2] conidiophores not abundant, simple, directly on the hyphae or as slightly modified hyaline branches varying in length; conidia borne in a cluster at the denticulate tip of the conidiophore, hyaline, elongate ovoid to broadly ovoid, somewhat narrowed at one end, 4–10 × 2–5 μ. In the cultures studied, there were numerous globose spores or chlamydospore-like cells, 5–7 μ in diameter, but their exact relationship to the cultures was not determined.

Cultures from bark of dead or dying subalpine fir collected in the Rocky Mountain National Park, September 9, 1955, F.P. #70803, and from bark of similar trees near Durango, Colorado, collected October 1955, have not developed perithecia in abundance. Their development is so slow that it is difficult to establish good herbarium specimens. Perithecia are present in considerable abundance in beetle tunnels of *Dryocetes confusus* but are often not in good condition to show ascospores; also they are usually mixed with smaller perithecia of other species. The writer had detected no *Leptographium* imperfect stage with this fungus, but Dr. Hunt suggests this species.

Ceratocystis nigra sp. nov.[3]

Perithecia in culturis evoluta, rugosa vel glabra, atra, 100–135 μ in diam., subsphaerica; rostra atra, 170–250 μ longa, ad basim 18–30 μ, ad apicem 12–14 μ in

[2] Growth measurement throughout this paper is diameter of mat in 5 days on 2% diamalt agar medium.

[3] The Latin descriptions were prepared by Miss Edith K. Cash, Mycologist, National Fungus collections, Plant Industry Station, Beltsville, Md.

diam., apicibus conicis, hyphis brevibus hyalinis irregularibus ornatis; asci evanidi; ascosporae in cirrhis extrusae, hyalinae, cylindricae usque bacillariformes, 3–4.5 × 1.4–1.8 μ; conidiophora hyalina, brevia, simplicia vel ramosa, plerumque 10–30 μ longa; conidia late ovoidea usque anguste cylindrica, vulgo ad apicem unum attenuata, 3–7 × 1.2–2.4 μ.

Perithecia developed in cultures, rough to smooth, black, small, 100–135 μ diameter, spherical or with height slightly greater than width; necks black, 170–250 μ long, 18–30 μ in diameter at the base and 12–14 μ at the top; tip of necks conical but with no regular hyaline ostiolar hyphae, sometimes a few irregular short hyaline hyphae at the tip of the neck; asci indistinct but spores in groups of 8; ascospores extruded in very narrow cirrhi, hyaline, cylindrical to rod shaped, 3–4.5 × 1.4–1.8 μ.

Cultures hyaline to white, medium growth rate (about 18 mm diameter), remaining white with sparse surface growth; perithecia black, developing slowly and maturing in about 4 weeks; conidiophores hyaline, short, simple or branched, mostly 10–30 μ long; conidia variable in size, grouped at top of conidiophore, broadly ovoid, to narrow cylindrical—usually narrower at one end, 3–7 × 1.2–2.4 μ.

The first culture of this species was isolated from canker-like streaks in the bark of subalpine fir, collected in the Rocky Mountain National Park in Colorado, September 9, 1955, F.P. #70805. Perithecia developed in a culture of a faster growing dark *Leptographium* sp. Unmixed cultures were obtained from perithecia in galleries of bark beetles from the Rocky Mountain National Park trees and also from similar bark beetle galleries collected from subalpine fir near Durango, Colorado, November, 1955 (TYPE specimen #70804).[4] Older cultures develop fewer perithecia, or perithecia may start to develop but fail to mature.

Ceratocystis brunnea sp. nov.

Perithecia atro-brunnea usque atra, sphaerica, 100–120 μ in diam.; rostra atra, 400–500 μ longa, ad basim circa 35 μ in diam., apicem versus usque at 12 μ attenuata, hyphis ostiolaribus hyalinis, 17–28 μ longis, attenuatis ornata; asci non visi; ascosporae hyalinae, in cirrhis angustis emergentes et ad apicem rostri aggregatae, utrinque subacutae, leniter curvatae, 4–5.2 × 1.5–2 μ; conidiophora brevia, hyalina; conidia hyalina, ad apicem conidiophori agglutinata, elongata vel late cylindrica, 3–5 × 2–3 μ.

Perithecial primordia numerous, on dark brown surface mat, mostly not maturing, few perithecia in old cultures developing necks and finally ascospores. Perithecia dark brown to black, spherical, 100–120 μ in diameter; sometimes clustered, necks black, 400–500 μ long, about 35 μ diameter at base tapering to 12 μ at top; ostiolar hyphae hyaline, 17–

[4] Type specimens have been deposited with the National Fungus Collections, Plant Industry Station, Beltsville, Maryland.

28 μ long, tapered to a point, somewhat spreading; asci not seen; ascospores hyaline, emerging in narrow cirrhi, narrow and somewhat pointed at the ends and slightly curved, 4–5.2 × 1.5–2 μ.

Cultures soon turning dark brown, with appressed hyphae, very little aerial mycelium; slow to medium growth (18 mm diameter), perithecia developing slowly, maturing in 4 to 5 weeks; conidiophores short branches on undifferentiated hyphae, hyaline; conidia hyaline, clustered at tip of conidiophores, elongate or broadly cylindrical but narrowed slightly to attached end, 3–5 × 2–3 μ.

Cultures of this species were isolated from perithecia in insect galleries in subalpine fir, the same material from which the previous two species were obtained—Rocky Mountain National Park, September 9, 1955, and near Durango, Colorado, November 1955 (TYPE specimen #70806).

The species differs in the brown color of mycelium, appressed type of growth, and in perithecial characters—including the hyaline hyphae around the ostioles. The usual failure to develop normal perithecia on the malt agar medium makes this fungus difficult to work with, but cultures are distinct from those of other fungi found here.

CERATOCYSTIS MINOR (Hedgc.) Hunt

This is a common species in bark of subalpine fir, where it was not definitely associated with insects. It was isolated several times from logging wounds in second growth subalpine fir on the Fraser Experimental Forest near Fraser, Colorado. In the same area it was observed in dead fir trees, where it sometimes developed as extensive dense black patches of sclerotia and perithecia between bark layers. It was collected on the bark of dead firs in the Rocky Mountain National Park September 9, 1955, and near Durango in November 1955. It has also been collected on dead or dying lodgepole pine, *Pinus contorta* Dougl.

Cultures are somewhat different from those studied from pine in the eastern part of the country. The mycelium is much lighter and with more abundant aerial (almost white) mycelium—not appressed as described by Hunt (**7**). Perithecial primordia (small brown spherical bodies) begin to develop in the white mycelial mats but have not been observed to mature or to develop ostiolar necks and ascospores. Growth is rapid, as in eastern cultures.

This species as described by Hunt (**7**) needs further investigation of distribution, host and insect relationships, and taxonomy. Rumbold (**8**) studied numerous isolates from eastern and western sources and noted certain differences, but considered only those from *Dendroctonus pscu-*

dotsugae (**9**) (*C. pseudotsugae*) as a distinct species. The form associated with *D. pseudotsugae* is present in the central Rocky Mountain area but has not been compared with the cultures from subalpine fir by the writer.

SPECIES FROM ENGELMANN SPRUCE INFESTED WITH BARK BEETLES

In the recent paper (**5**), several species of Ophiostomataceae were described as being fairly closely associated with *Dendroctonus engelmanni* Hopk. Further isolations from associated bark beetles, apparently secondary in *Picea engelmannii* Perry, have yielded additional species of fungi belonging in this group.

Ceratocystis bicolor (Davidson and Wells) comb. nov.

Ophiostoma bicolor Davidson and Wells, Mycologia **47**: 63. 1955.

This species has repeatedly been isolated from *Ips pilifrons* adult beetles infesting bark of Engelmann spruce killed by *D. engelmanni*. It seems to be the principal fungus associated with this *Ips* beetle, but the isolation samples used were from limited distribution.

Ceratocystis perfecta sp. nov.

Perithecia abundantia, atra, ad basim globosa et subhyalina, tenui-tunicata, glabra, 80–130 μ in diam.; rostra atra, glabra, 200–400 μ longa, ad basim, 15–25 μ, ad apicem 11–15 μ in diam., hyphis ostiolaribus 7–12, hyalinis, 16–20 μ longis, attenuatis ornata; ascosporae in globulam hyalinam ad apicem rostri agglutinatae, allantoideae, 4–5.5 × 1.5–2 μ; conidiophora brevia, hyalina, simplicia; conidia hyalina, cylindrica, utrinque subattenuata, curvata vel fere recta, 9–12 × 2–3 μ.

Perithecia developing quickly and in great abundance, bases globose, black under the binocular dissecting microscope but lower part almost hyaline, under high power, with upper side (around base of neck) black, thin walled, smooth, 80–130 μ in diameter; necks black, smooth 200–400 μ long, 15–25 μ in diameter at base, 11–15 μ at tip; 7–12 hyphae around ostiole, hyaline, 16–20 μ long, narrowed to tips; ascospores collecting in a spherical hyaline droplet at tip of necks, allantoid, 4–5.5 × 1.5–2 μ.

Cultures remaining hyaline, with surface smooth or covered with abundant massed conidia; growth slow, about 8 mm diameter; perithecia forming and maturing within a 2-week period—in dense clusters; conidiophores short hyaline, simple; conidia hyaline, cylindrical and slightly tapered at ends, curved to almost straight 9–12 × 2–3 μ.

Developing in a mixed culture from *Ips pilifrons* adult beetle from Engelmann spruce log in laboratory, Fort Collins, Colorado, January 1956 (TYPE #70808).

Ceratocystis tenella sp. nov.

Perithecia abunde in culturis evoluta, atra, ad basim sphaerica, 120–140 μ in diam., interdum spinis brevibus obscuris ornata; rostra atra, 350–500 μ longa, ad basim circa 25 μ, ad apicem 12–15 μ in diam., hyphis ostiolaribus 10–15, 30–45 μ longis, ad basim 2–2.5 μ in diam., apicem rotundatum versus leniter attenuatis praedita; ascosporae hyalinae, subcurvatae, 3.5–5 × 1.3–1.7 μ; conidiophora brevia, hyalina; conidia late ovoidea usque elongata, interdum subcurvata et ad apicem unum attenuata, 2.5–6 × 1.2–2.5 μ, non abundantia.

Perithecia abundant and developing quickly in culture, black throughout, base spherical, 120–140 μ diameter, occasionally few short dark spines, necks black, 350–500 μ long, about 25 μ diameter at base and 12–15 μ at the tip; 10–15 hyaline hyphae around ostiole, 30–45 μ long, 2–2.5 μ in diameter at base, tapering slightly to rounded tip, spreading outward; ascospores hyaline, slightly curved (probably arranged symmetrically in spherical asci), 3.5–5 × 1.3–1.7 μ (probably shaped nearly like orange segments).

Cultures slow to medium growth (about 20 mm diameter) developing some dark color—in patches—; perithecia usually abundant and maturing in 2–3 weeks, sometimes clustered with white tufted to cottony surface mycelium; conidiophores short branches of vegetative hyphae, hyaline; conidia variable in size and shape from broadly ovoid to elongate, sometimes slightly curved and narrower at one end, 2.5–6 × 1.2–2.5 μ, not in great abundance.

Isolated from small secondary bark beetles associated with *Dendroctonus engelmanni* and *Ips pilifrons* in Engelmann spruce logs, January 31, 1956 (TYPE specimen #70809).

Ceratocystis vesca sp. nov.

Perithecia in culturis abundantissima, atra, sphaerica, 75–110 μ in diam., glabra, tenui-tunicata; rostra atra, fere cylindrica, 300–400 μ longa, ad basim 20–25 μ, ad apicem 18–25 μ in diam., hyphis ostiolaribus brunneis, ad basim 2.5 μ, ad apicem 1.5 μ crassis, 75–125 μ longis ornata; ascosporae in cirris longis succineis extrusae, hyalinae, irregulariter galeiformes, 3–3.5 × 1.5 μ, ad apicem unum leniter latiores et rotundiores; status conidiophorus typi *Graphii*; stipites usque 220 μ alti, 10–20 μ lati, atro-brunneis, ad apicem pallidiores; conidia in massam mucosam ad apicem conidiophori agglutinata, cylindrica usque ovoidea, 2–6 × 1.8–3 μ.

Perithecia in culture very abundant, black, spherical, small, 75–110 μ in diameter, smooth, thin-walled; necks black, nearly cylindrical, 300–

400 μ long, 20–25 μ in diameter at base, and 18–25 μ at top, when mature; ostiolar hyphae are continuous with neck hyphae, brown, 2.5 μ thick at base to 1.5 μ at tip and 75–125 μ long, upright or bristle-like; ascospores extruded in long hair-like amber-colored cirrhi, hyaline, irregularly hat-shaped, 3–3.5 × 1.5 μ, slightly broader and more rounded at one end.

Cultures medium fast-growing, with *Graphium* stage developing in great abundance first, then perithecia developing in abundance interspersed with the coremia in 4 or 5 days, maturing in 10-day-old cultures; conidial stage typical of modified *Graphium* in 4–7 days, coremia small to medium large, up to 220 μ high and with stalks 10–20 μ thick, light brown to dark brown at base and light brown at top with conidia in sticky mass at top; conidia developing in a few days on simple conidiophores, hyaline; *Graphium* fruiting stalks (with few intermediate *Leptographium*-like conidiophores), with conidia cylindrical to ovoid, 3–6 × 1.8–3 μ.

Isolated from secondary bark beetle associated with *Ips pilifrons* and *Dendroctonus engelmanni* in Engelmann spruce logs, January 31, 1956 (TYPE specimen #70807).

The strain of *C. vesca* under study here is a very prolific-fruiting one in contrast to somewhat related species that have been studied, such as *C. obscura* (Dav.) Hunt and *Ophiostoma truncicola* Davidson. Perithecia are smaller than in related species and ascospores are different in shape and size.

ASPEN SPECIES

The species CERATOCYSTIS MONILIFORMIS (Hedgc.) Moreau was collected several times. It fruited on freshly cut aspen sapwood during rainy periods, near Mancos, Colorado, July 1954, and near Norwood in June 1954. Perithecia developed on wood, and in 3 or 4 days in cultures, were characteristic of this fast-growing species.

An undescribed species from ambrosia-beetle galleries in aspen trees has been studied and described as follows:

Ceratocystis brevicollis sp. nov.

Perithecia interdum in massis densis evoluta, sphaerica, primum hyalina pallidave, demum atra, glabra, 150–180 μ in diam.; rostra fere cylindrica, atra, 200–300 μ longa, ciliis ad ostiolem conicum dispositis; ascosporae hyalinae, subcurvatae, 4.5–5 × 1.3–2 μ, plerumque vagina gelatinosa inconspicua ad laterem unum affixa; conidia hyalina, globosa vel ovoidea, 3–7 × 2–3 μ, e myeclio vel conidiophoris parvis hyalinis oriunda, aut hyalina, elongata, 3–9 × 1.4–3 μ, e conidiophoris typi *Leptographii*, pallide brunneis, septatis, penicilliter ramosis, ad basim 5–9 μ latis, sursum attenuatis hyalinisque oriunda.

Perithecia starting to develop in about 3 weeks, hyaline or only slightly colored at first, maturing in 4 to 5 weeks, sometimes in dense masses, becoming perfectly spherical, smooth, black, 150–180 μ in diameter, with necks almost cylindrical, black, 30–40 μ thick and 200–300 μ long, with bristles at the tip usually conically arranged over the ostiole; ascospores hyaline, slightly curved, 4–5.5 × 1.3–2 μ and usually with inconspicuous gelatinous sheath attached to one side.

Cultures slow-growing, 8–10 mm diam., mycelium remaining white to light gray, appressed, sparse, scarcely visible on the surface of the culture medium; conidia hyaline, borne directly on the mycelium or on small hyaline slightly differentiated conidiophores, globose to ovoid, 3–7 × 2–3 μ, or on special *Leptographium*-like conidiophores, hyaline, elongate (cylindrical to slightly ovoid but usually broader at one end), 3–9 × 1.4–3 μ; *Leptographium* conidiophores with light brown stalks, septate, penicillately branched at tip (with conidia in mucus mass), 5–9 μ thick at base and narrowed above, hyaline at tip.

In ambrosia-beetle (*Trypodendron retusus* Lec.) galleries in trunks of recently killed aspen with bark still on, Roosevelt National Forest, along Cache La Poudre River, near Fort Collins, Colorado. The insects were collected and identified by T. O. Thatcher, Entomologist, Colorado State University, and the fungus isolated in September 1954 (TYPE specimen #70810) by Carl Seliskar, then Pathologist, Colorado State University, and now Chief, Forest Disease Research, Central States Forest Experiment Station.

This fungus differs markedly from any species known to the writer in that the mycelium is almost hyaline and growth is slower than that of any of those associated with bark beetles except *C. perfecta*. Perithecia are more nearly like those described for *C. obscura* (David.) Hunt (1) except that ostiolar filaments are more nearly similar to those of *C. minuta* (Siem.) Hunt (2) but are slightly longer. The conidiophores are inconspicuous and seem to develop along with the perithecia or slightly after the perithecia mature.

No survey has been made to determine whether this fungus is consistently associated with the beetles, or whether it is common and widespread in this area.

DISCUSSION

It was stated in the introduction that additional information is needed to clarify the host and insect relationships of the western wood-staining fungi. However, it may be helpful to point out for future investigators some general relationships appearing at present.

Rumbold (**8**) showed that a common *Dendroctonus* in pines in the east has the fungus *C. minor* (*C. pini*) consistently associated with it. In the west *C. minor* is common to abundant in pines and subalpine fir. However, it does not appear to be associated with any species of *Dendroctonus*. It is probably distributed by other insects, as yet undetermined. Possibly there may be differences between the so-called eastern and western *C. minor*. If the form associated with *D. pseudotsuga* (**9**) in Douglas fir in Colorado is considered a form of *C. minor*, as suggested by Hunt (**7**), it is an exception to the above statement regarding *Dendroctonus* association.

As pointed out by Rumbold (**10**), *C. montium* seems to be the principal fungus associated with mountain pine beetle (*D. monticolae* Hopk.) and Black Hills beetle (*D. ponderosae* Hopk.). A *Leptographium* sp. has been isolated from trees killed by the Black Hills beetle, from pine attacked by turpentine beetle (*D. valens* Lec.) in Nebraska, and from western white pine attacked by *D. monticolae*. However, it seems probable to the writer that in some of these cases, if not all, *Leptographium* was carried in by secondary beetles. *Dendroctonus convexifrons* in *Pinus ponderosa* from Colorado has a *Leptographium* sp. closely associated with it. It is known that species of *Dendroctonus* in spruce (**5**) carry species of *Leptographium* in close association. The *Leptographium* commonly isolated from fir in association with several species of *Ceratocystis*, described in this paper, has not been definitely connected with a specific bark beetle.

The *Leptographium* species here in the Rocky Mountain area have not been described or identified to species, except for the one in Engelmann spruce (**5**). None of the cultures has had a perithecial fruiting stage develop in it and none of the perfect stages on the host tissue from which *Leptographium* sp. was isolated has proved to be connected with this conidial stage.

No *C. ips* culture has been isolated from *Ips pilifrons* in spruce, although *C. bicolor* is associated with this beetle in the same tree. It is possible that *C. ips* may be associated with *Ips* sp. in pines, but they have not been studied here.

The several publications referred to mention certain *Ceratocystis*-insect associations and in many instances there are close connections between them. This does not or should not imply that a particular insect does not have other fungi, yeasts, bacteria, etc., that are also associated. In the insect galleries in subalpine fir, three perithecial species and *Leptographium* were isolated from a small area of the infested bark. In bark of Engelmann spruce (**5**) several species besides *Leptographium* were present in close association with *Dendroctonus*

engelmanni galleries. The presence of some of these organisms could be due to secondary insect invaders, but isolation methods have not been sufficiently developed or of an intensity designed to show detailed associations. It would be desirable if future studies could show in greater accuracy some of the interesting insect fungus relationships, from the standpoint of both their biological and physiological aspects.

SUMMARY

Four species of Ophiostomataceae have been isolated from dead or injured subalpine fir (*Abies lasiocarpa*). Two (*C. nigra, C. brunnea*) are described as new species associated with bark beetle galleries, and two (*C. minor, C. penicillata*) are species previously reported as associated with bark beetles.

Three other new species are described from secondary bark beetles associated with *Dendroctonus engelmanni* in *Picea engelmannii*. These are *Ceratocystis perfecta, C. tenella,* and *C. vesca.*

Two species are reported from aspen (*Populus tremuloides*) sapwood in Colorado for the first time. *C. brevicollis* is a new species associated with ambrosia beetles in aspen.

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STUDIES ON MICROSTRUCTURE OF LARICIFOMES OFFICINALIS

ALCIDES RIBEIRO TEIXEIRA

(WITH 6 FIGURES)

In studying the microstructures of fomitoids that might be considered closely related to *Fomes fomentarius*, I came across the well known *Fomes officinalis*. Specimens of this fungus are quite remarkable in their construction, and very different from *fomentarius* or any other species of the genus *Fomes* sensu str. In the first place, the hyphae of *officinalis* are always hyaline, giving the characteristic white color of the context, never present in any species of typical *Fomes*. Second, the typical crust, which forms the surface of all species of *Fomes*, is absent in *officinalis*, whose surface is constituted of a very simple cortex, which has a microscopic trichoderm in younger portions and then becomes glabrous, slightly hardened, sometimes even rimose.

The character of the hyphae, whether hyaline or colored, and the construction of the covering of the surface of the pileus are of prime taxonomic value in the segregation of genera of polypores; and because of the striking differences in micromorphological characters between specimens of *fomentarius* and *officinalis*, these two species ought not to be considered congeneric. Therefore I decided to describe a new genus for *officinalis*. The drawings were made and the manuscript was being completed when (on Oct. 28, 1957) the Library of the N. Y. Botanical Garden, where I had been working, received fascicle 3 of vol. 11 of Česká Mykologie, with the article by Kotlaba and Pouzar (**1957**) "Notes on Classification of European Pore Fungi," in which these authors described the new genus *Laricifomes*, based on *Polyporus officinalis* (Vill. ex Fr.) Fries.

However, for the purpose of this particular research, in which characteristics of the microstructures of the carpophore are considered of greatest taxonomic importance in the segregation of genera of the Polyporaceae, the generic description given by Kotlaba and Pouzar (l.c., p. 158) is not quite complete. Characters such as the construction of the surface of the pileus, peculiarities of the hyphal walls, whether the differentiated hyphae (which they referred to as "tertiary") are of limited

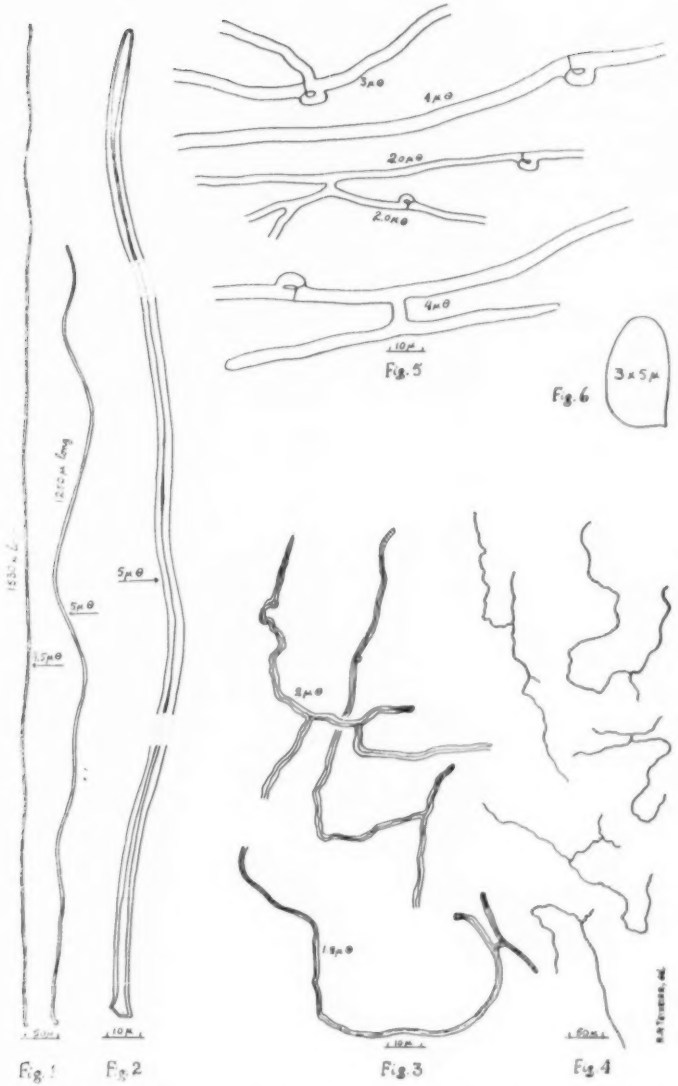
growth, presence or absence of cystidia, basidial characteristics, and type of spores, were not taken into consideration by those authors in their description of the genus. On the other hand, many characters given by them as of generic rank are here considered as merely specific: for example, the character of being annual or perennial (known to be a very inconstant one when temperate and tropical specimens of the same species of pore fungi are compared), the shape of the fruiting body (also very variable for most species according to conditions of growth), and the fragility of the carpophore when dry.

Studying the most important literature on *officinalis*—especially Calkins (**1886**), Peck (**1903**), Ames (**1913**), Murrill (**1907–14**), Neuman (**1914**), Lloyd (**1915**), Faull (**1916**, **1919**), Bourdot & Galzin (**1928**), Donk (**1933**), Pilát (**1936–42**), Lohwag (**1940**), Bondarzev & Singer (**1941**), Lowe (**1934**, **1942**, **1957**), Nobles (**1948**), Overholts (**1911**, **1953**), and Bondarzev (**1953**), one can note several discrepancies in the descriptions of the microstructures of the species. While Nobles (**1948**) and Faull (**1916**) described the hyphae as septate and possessing clamp-connections, others, including the quite recent work of Overholts (**1953**), stated that the hyphae were without septa! That means that most of those authors had never seen the generative hyphae in specimens of this species. These hyphae are hyaline, frequently branched and with conspicuous clamp-connections. The surface of the pileus has also been described differently by several authors. Some have described it as covered with an indistinct crust (e.g., Pilát, l.c.); others have said that the surface is not encrusted (Ames, l.c., for instance); while several stated that the surface is a crust similar to that of *fomentarius, applanatus,* etc. The only one of these authors who made any special effort to determine the real construction of the surface of specimens of *officinalis* was Lohwag (**1940**), who found that the surface of the pilei is formed by a cortex, and not by a crust. This has also been confirmed in this study. The covering is in fact a very simple cortex, as it is described below.

The description of the species, embodying the results of the necessary study of the microstructures and of the construction of the carpophore of *officinalis,* is here extended and corrected.

LARICIFOMES OFFICINALIS (Vill. ex Fr.) Kotl. et Pouzar, Česká Mykol. **11**: 158. 1957.

Syn.: Polyporus officinalis (Vill.) ex Fries, Syst. Myc. **1**: 365. 1821; *Ungulina officinalis* Fr. ex Pat., Ess. Tax. 103. 1900; *Fomes albogriseus* Peck, Bull. Torr. Bot. Club **30**: 97. 1903; *Fomes laricis*



FIGS. 1–6. *Laricifomes officinalis.* FIGS. 1, 2. Acicular (skeletal) hyphae from the context and dissepiments. FIGS. 3, 4. Complex, branched, differentiated (binding) hyphae, common in the context but rare in the dissepiments. FIG. 5. Generative hyphae, showing types of branching, and clamp-connection. FIG. 6. Spore.

Jacq. ex Murrill, Bull. Torr. Bot. Club **30**: 230. 1903; *Fomes officinalis* (Vill. ex Fr.) Faull, Roy. Canad. Inst. Trans. **11**: 185. 1916.

CARPOPHORE sessile, becoming ungulate, with indefinite growth, specimens commonly found to measure about 5 × 20 cm, some recorded as being more than 60 cm long (Neuman, **1914**). CONTEXT white and cheesy when fresh, becoming discolored, rigid and friable when dry or old, 2–5 cm thick; formed mostly by hyphae which are differentiated branches of generative hyphae. The generative hyphae are better seen in the growing margin of the pileus, because they soon collapse and disappear; they are delicate or thin-walled, little branched, 2.5–3.5 μ diam., some as wide as 4 μ, and some as narrow as 2 μ, with simple and conspicuous clamp-connections; a few H hyphae can be found (FIG. 5). The differentiated branches, which form most of the context, and never show clamp-connections, are of two kinds: a) aciculiform skeletal hyphae (FIGS. 1, 2), hyaline, thick-walled to subsolid, unbranched, aseptate, of limited growth, up to 1500 μ long, some as short as 600 μ and a few as long as 1900 μ, flexuose, growing longitudinally in the flesh, interwoven, rather fragile, 3–4.5 μ diam., some as thin as 2.5 μ, others as thick as 5 μ; b) binding hyphae (FIGS. 3, 4), also hyaline, mostly subsolid to solid, frequently branched, branches short, up to 300 μ long, rarely up to 450 μ or more, also fragile, averaging 1.8–2.2 μ diam. The hyphae are immersed in an amorphous substance, easily seen when mounted in methylene blue, but almost disappearing when mounted in KOH-phloxine, because dissolved by the potash solution. This amorphous substance comes out in one's fingers as a very fine whitish powder. The context tastes slightly sweet, then bitter. SURFACE whitish, glabrous, concentrically sulcate according to the layers of tubes, sometimes becoming grayish with age, slightly encrusted and even subrimose; the structure is a simple cortex, the hyphae of the context (the differentiated branches of the generative hyphae) approach the surface without any further differentiation, becoming gradually more densely interwoven, but not forming any sharp contrasting layer; they reach the surface obliquely, forming a microscopic trichoderm which disappears when that part of the pileus becomes older, giving a smooth appearance to the cover. With age the covering formed by the dense matting of hyphae may become rimose, as happens in other species with similar (even though much harder) kinds of surface—e.g., species of *Phellinus*. TUBES normally of the same color and consistency as the context, commonly 5–10 mm long each season, sometimes up to 20 mm long (Overholts, **1953**), distinctly stratified, once recorded as having about 70 strata (Neuman, **1914**). The pores are angular, with thick edges, averaging 3–4 per mm. The hyphae of the dissepiments are very similar to those of the context, with very few of the branched differentiated ones (binding hyphae) present. Some of the unbranched differentiated hyphae (skeletal) are almost 2 mm long (1730 μ, 1880 μ), all of them running longitudinally,

loosely interwoven, fairly easy to tease apart, in spite of being quite brittle. Only in the youngest layers can one find tubes with lumen free of plugging hyphae. In the older layers all tubes are full of hyphae, the oldest tubes having almost completely disappeared, making it difficult to separate them from the context. HYMENIUM—Basidia normally clavate, originating from clamped generative hyphae. Cystidia absent. Spores hyaline, smooth, delicate-walled, not amyloid, averaging 3–4 × 4–5 μ (FIG. 6).

After a careful study of the anatomy of the carpophore and morphology of the microstructures of specimens of *officinalis*, and considering that the original description of the genus *Laricifomes* (based mostly on external morphology of the fruit body) is too narrow in the sense of the external morphology and too broad as far as the characters of the microstructures are concerned, the description of that genus is here modified:

LARICIFOMES Kotl. and Pouzar, Česká Mykol. **11**: 158. 1957.

Pileus commonly stratose-perennial. Cover of the surface constituted by a cortex. Context white to isabelline. Generative hyphae hyaline, with clamp-connections, producing two different kinds of specialized branches, both always hyaline: a) unbranched, of limited growth, flexuose, growing longitudinally in the flesh, thick-walled to subsolid; b) freely branched, branches rather short, mostly solid. Cystidia absent. Basidia clavate. Spores hyaline, smooth, delicate-walled, not amyloid.

TYPE: *Laricifomes officinalis* (Vill. ex Fr.) Kotl. et Pouz., l.c. *Syn.: Polyporus officinalis* (Vill.) ex Fries, Syst. Myc. **1**: 365. 1821.

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OXIDATION OF FATTY ACIDS BY CELL SUSPENSIONS OF SPICARIA VIOLACEA [1]

G. T. JOHNSON

Spicaria violacea can grow in a mineral-salts solution with saturated fatty acids as the sole carbon source. Since the growth response varies with the different acids (**5**), attempts have been made to test the effect of these substrates on O_2 uptake by the mold. The organism was grown in a Czapek-Dox medium (temperature, 30° C; pH, 7.2; carbon source, 5% glucose or glycerol; other conditions, as indicated below). Cultures were harvested by filtration, ground in a mortar with distilled water, pressed through coarse cheesecloth, and washed by centrifugation. The sediment was then taken up in distilled water to form a cell suspension which was then used for O_2-uptake studies employing conventional manometric techniques.

Preliminary experiments indicated the optimum temperature for endogenous O_2 uptake by glucose-grown cells to be approximately 30° C; optimum uptakes were obtained at slightly higher temperatures with cells grown on glycerol. Glucose-grown cells showed high and fairly uniform endogenous rates between pH 5.8 and 7.2; glycerol-grown cells gave highest readings in a slightly alkaline range (7.2–7.6). Hence the fatty-acid effects were examined at a temperature of 30° and a pH of 7.2. Each Warburg vessel contained 1.0 ml cell suspension and 1.0 ml 0.2 M phosphate buffer, pH 7.2. The side arms contained 0.8 ml 0.1 M substrate (pH 7.2) and the center well 0.2 ml 6N NaOH. Substrates were commercial preparations of the highest available purity. The fatty acids were converted to the potassium soap with KOH in absolute alcohol, precipitated and washed with anhydrous ether, and dried in a vacuum desiccator. Cups were oxygenated for three minutes and equilibrated for fifteen minutes before the substrate was tipped into the main well.

[1] This investigation was supported in part by a grant-in-aid from the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council. It includes some work done while a Research Participant, Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tennessee, under contract with the United States Atomic Energy Commission.

TABLE I presents the effect of saturated fatty acids (C_2 to C_{18}, except C_{15}, which was not available) on the O_2 uptake of *Spicaria violacea*. All acids with chain lengths from C_2 to C_7 and C_{16} to C_{18} gave a stimulatory effect, with palmitate (C_{16}) providing the most striking response. Fatty acids of chain lengths from C_8 to C_{14} inhibited O_2 uptake, with some, e.g., caprate (C_{10}), undecanoate (C_{11}), and laurate (C_{12}), yielding values considerably below the endogenous rate.

TABLE I

EFFECT OF FATTY ACIDS ON O_2 UPTAKE OF SPICARIA VIOLACEA†

Substrate	Time—Minutes			
	60	120	180	240
Stearate	2.8	4.0	7.0	10.3
Margarate	3.6	5.8	9.6	13.9
Palmitate	4.0	7.9	14.0	21.7
C_{14}–C_8 Substrates	0.0*	0.0*	0.0*	0.0*
Heptate	2.6	4.3	7.5	11.4
Caproate	2.1	4.8	9.8	16.9
Valerate	1.8	3.6	6.8	10.5
Butyrate	1.0	1.5	2.3	3.0
Propionate	0.6	0.6	1.0	1.0
Acetate	1.1	2.3	3.6	6.1
Glucose	2.0	3.2	6.6	9.8
Glycerol	0.6	1.3	2.1	3.6

† Four day glucose growths from submerged culture. Endogenous subtracted. Data indicate μl O_2 uptake/mg dry wt. for periods shown. Cup contents and conditions indicated in the text.

* 0.0 = less than endogenous values.

O_2-uptake measurements were made on both glucose and glycerol growths from submerged cultures of four different ages (1 to 7 days). In all eight experiments data similar to those in TABLE I were obtained. No differences were noted between glucose and glycerol growths except in their respective responses to glucose and glycerol. Glycerol growths gave far higher O_2-uptake values on glycerol than on glucose; glucose growths gave much higher readings on glucose than on glycerol. Data from submerged cultures on Sabouraud's medium (two experiments, 3 and 5 day growths) were similar to those for glucose growths. In all ten experiments palmitate gave the greatest stimulatory O_2-uptake response.

Considerable grinding was required to separate the filaments from surface mats, but most of the suspension represented intact cells. Four experiments using glucose-grown surface growths of different ages (14 to 28 days) and four more using similar preparations grown on glycerol

(ages 10 to 28 days) also repeated the general pattern described above. In some cases a slight lag occurred (perhaps due to the greater age of the cells) before higher readings were obtained with the stimulatory substrates. In these eight experiments with surface-grown cells palmitate also gave the greatest O_2-uptake response. In general, the older the growth, the more striking the palmitate response appeared when compared with endogenous rates.

Fatty acids serve as the sole carbon source for some fungi (**5**). With others these substrates inhibit growth (**9, 14**), stimulate O_2 uptake (**1, 7, 8**), inhibit O_2 uptake (**7, 8, 11**), and inhibit fermentation (**10**). In some cases fatty acids apparently serve as growth factors or growth factor substitutes, as in *Neurospora crassa* (**3, 6**) and several bacteria (**2, 4, 12, 13**).

Specific fatty acids markedly stimulate O_2 uptake by *Spicaria violacea*. With C_2–C_7 and C_{16}–C_{18} acids (in particular with C_6 and C_{16}) the stimulation is significantly above the levels given by favorable substrates like glucose or glycerol, on which the cells tested had been grown. These effects are reproducible under a variety of conditions, with cells of different ages, from different media, and grown under varying O_2 supply. Fatty acids of chain lengths from C_8 to C_{14} inhibit O_2 uptake under the same conditions. Thus, to a certain degree, the chain length of the fatty acid is associated with its action on respiration; some fatty acids (C_2–C_7 and C_{16}–C_{18}) stimulate and some (C_8–C_{14}) inhibit O_2 uptake by the mold. With the exception of myristate (C_{14}), the acids which stimulate O_2 uptake are those which provide for growth (**5**), and those which inhibit O_2 uptake are those which provide unsatisfactory substrates for this organism. Further work is in progress designed to clarify possible mechanisms behind the apparent chain-length correlation with the action shown.

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DEUTEROMYCETES I: THE SPORIDESMIUM COMPLEX [1]

ROYALL T. MOORE

(WITH 17 FIGURES)

An attempt to identify a *Sporidesmium* leads one to the realization that such an identification is at present impossible. Researches toward a monograph of the genus have made manifest that before such a compilation can be completed, a disposition of the plethora of genera implicated must be attempted. The genus *Sporidesmium* [2] as it is generally understood has ellipsoid, phaeodictyous conidia borne on simple conidiophores (and thereby distinguished from *Stemphylium* [3]). The name has at one time or another been applied to nearly 250 fungi, many of which are obviously members of other genera. However, those with conidia as described above fall into three groups on the basis of colony type: acervulate, sporodochial, and (the majority) in the Dematiaceae. Within this broad definition the spores vary tremendously in shape, size, production, attachment, wall thickness, etc. The interplay of these char-

[1] A portion of a thesis to be submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Harvard University.

[2] Though the spelling *Sporodesmium* is frequent and sanctioned by "A Dictionary of the Fungi," it is counter to Art. 73 Note 1 of the Code of Nomenclature (Paris); Art. 73 Note 2 and Rec. 73G(d) do not apply as the etymology is from the Greek σπορα + δεσμε.

[3] In a personal communication, E. G. Simmons characterizes *Stemphylium* as follows:

"In devising a strict concept of the genus [*Stemphylium*] I would insist on a type of conidial production admirably illustrated by Wiltshire [**16**] in his fig. p. 226. The first conidium always is produced as a protoplasmic extension through a pore in the tip of the conidiophore; this protoplasmic extension grows into a spore and produces its own spore wall without any connection with portions of the conidiophore itself except for the tiny protoplasmic thread running through the pore, *i.e.*, there is no continuity between the wall of the conidiophore and the wall of the conidium. If the protoplasmic thread breaks, the conidium falls away. In producing an additional spore, the conidiophore grows out through its own tip and produces a new spore as before. The sites of successive conidiophore growth are marked by collars (if the tip-scar is large) or by swelling (if the scar is small)."

Such a strict adherence to Wallroth's original delimitation is obligatory if *Stemphylium* is to be clearly distinguished from the genera discriminated in this paper.

acters is such that there are essentially only two courses of treatment. The analytical would create a number of small, rigidly circumscribed, and often monotypic units, while the synthetic would provide several large units with minimal disruption and maximal facility in the overall taxonomy. I have favored the latter, conservative, treatment, particularly since a "nomenclatural type is not necessarily the most typical or representative element of a taxon" (Art. 7, Code of Nomenclature).

THE ACERVULATE ANALOGUE

STIGMOPSIS Bubák

Bubák established this genus for *Stigmella celtidis* and *St. montellica*, neither of which he designated as the type. Since it has been impossible to locate any authentic material of his *Stigmopsis celtidis* in the known Bubák collections, I hereby designate *St. montellica* as the generic lectotype. However, examination of Bubák's type material for *Stigmopsis montellica* proves it to be staurosporous and to belong to the genus *Cheiromyces* as clarified by Damon (**4**). It is put in this genus rather than *Dictyosporium* as elucidated by Damon (**5**) on the basis of the conidia, which branch in more than one plane and which are not U-shaped.

Cheiromyces montellica (Sacc.) comb. nov. FIG. 17.

≡ *Stigmella montellica* Saccardo, Michelia **1**: 90. 1877.

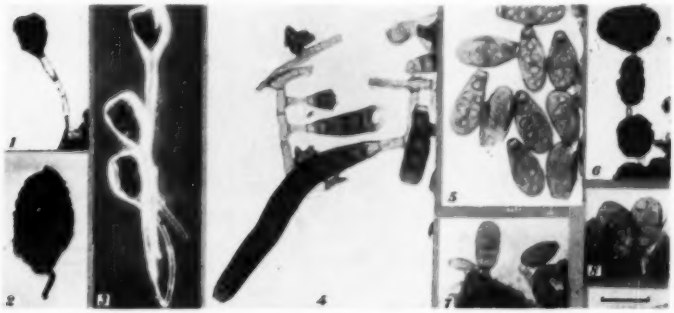
≡ *Stigmopsis montellica* (Sacc.) Bubák, Ann. Myc. **12**: 217–218. 1914.

Sporodochia dark, punctiform; conidia fuscous to opaque, sessile, 26–55 × 14.5–18.5(–26)μ, generally without a prominent basal cell, the digitations not uncommonly sub-branched, branches coattached though they may become centrally separated, 3–c.10-septate, septa becoming constricted.

Material examined: BKL (Bubák coll.), TYPE of *Stigmopsis montellica*, Bosco Montello, Italy, on *Salvia glutinosa*, leg. *Saccardo* (slide RTM I: 17). FH (Saccardo Myc. Veneta 1066), syntype of *Stigmella montellica*, Montello (Treviso), Italy, on living leaves of *Salvia glutinosa* (slide RTM I: 170); also present on this material is a *Ceratophorum* sp.

This species is readily distinguished by its large conidia, and it is further distinct, from *C. tinctus*, by the lack of a blue cast and a greater number of septa, and from *C. stellatus*, by the lack of conidiophores.

Three other genera have been credited with phaeodictyosporous acervuli: *Thyrostromella, Sciniatosporium,* and *Steganosporium.* The type species of each of the three is described as producing saprobic colonies ("in culmis et foliis siccis," "in ramulis . . . gelu necatis," and "auf abgestorbenen Aesten," respectively). *Thyrostromella* is an overlooked v. Höhnel (**7**) genus based on *Sporidesmium myrianum* Desm. that Hughes (**11**) reestablished in 1955 (FIGS. 7, 8). Examination of type and authentic material of the single species (NY, PC; slides RTM I:79, I:80, I:112, I:114), thoroughly described by Hughes, would indicate it to be congeneric with *Steganosporium.* Therefore, **Steganosporium myrianum** (Desm.) comb. nov.[4]



FIGS. 1, 3. *Piricauda paraguayense.* 1. Photomacrograph of conidium and conidiophore of *Spegazzini 3837.* 3. Darkfield photomicrograph showing the conidia and long tapering conidiophores, *Ule 948.* FIG. 2. *Berkleasmium conglobatum,* conidium showing terminal apiculus and stem-like conidiophore. FIG. 4. *Sporidesmium caricinum,* type material, showing the phaeophragmous conidia and the hyphopodia. FIG. 5. *Steganosporium pyriforme,* syntype ex Herb. UC, showing the very thick walls and the conspicuous mesial septa. FIG. 6. *Coniosporium olivaceum,* catenulus from type material. FIGS. 7, 8. *Steganosporium myrianum.* 7. Note thick basal scar of conidium on the right; to the left of the vertical conidium is a conidiophore cell showing conspicuous terminal thickening. 8. Older, muriform conidia. Both 7 and 8 also show the slightly echinulate nature of the conidia. (Scale line equals 25 μ.)

SCINIATOSPORIUM Kalchbrenner

Kalchbrenner issued material of this genus in 1866 as exsiccatus 985, *Sc. Lycii,* in Rabenhorst's *Fungi europaei exs.* and published it subse-

[4] ≡ *Sporidesmium myrianum* Desmazières, Ann. Sci. Nat. III **18**: 357. 1852. ≡ *Thyrostromella myriana* (Desm.) Höhnel, Ber. deut. bot. Ges. **37**: 157. 1919.

quently in Hedwigia in 1867. In 1875 Reinsch (**14**) described, but did not name, a second species. He made no mention of the author of the genus, nor was the name ever picked up Saccardo. However, Costantin (**3**) compiled the name and credited it to Reinsch, designating the unnamed species as *Sc. Hypnorum*. This has had the final and logical result of its being listed in the "Dictionary of the Fungi," 4th edition, as a Reinsch genus and being dismissed as a *nomen dubium*. Examination of good exsiccatum material shows erumpent colonies bearing short stipitate or sessile conidia that are initially phragmosporous; but there are present larger, presumably older conidia that are muriform. Therefore, I consider this genus to be a later synonym of *Steganosporium*.

STEGANOSPORIUM Corda
= *Sciniatosporium* Kalchbrenner
= *Thyrostromella* Höhnel

Acervuli subcuticular, subepidermal, or subcortical; conidia phaeodictyous, sessile or borne singly on short, simple conidiophores; paraphyses may be present.

TYPE: *Steganosporium pyriforme* Hoffm. ex Corda.

Steganosporium pyriforme Hoffm. ex Corda, Icones Fung. **3**: 22. 1839. FIG. 5.

Acervuli subcortical, saprobic, sterile paraphyses common among the sporogenous teeth; conidia fuscous, produced in great abundance and extruded onto the surface in black, gelatinous heaps, pyriform to clavate to obovate to cylindrical, 13–18.5(–21) × 26–40(–60.5) μ; spore walls very thick, multilaminate, septa often much thinner or perforate medially, the primary horizontal septa, particularly the mesial septum, often prominent; cell contents granular.

Material examined: Fries *Scler. succ.* 214, FH, UC, BPI, SYNTYPES (slides RTM I:74, I:151, I:152). Ex leg. H. L. Barnett, Morgantown, W. Va., on dead maple (RTM I: 153).

Steganosporium Lycii (Kalchbr.) comb. nov. FIG. 13.
= *Sciniatosporium Lycii* Kalchbrenner, in Rabenhorst, Fung. Europ. Exisc. II 10: 985. 1866, and Hedwigia **6**: 9. 1867.

Acervuli subepidermal, saprobic; conidia subglobose to oval, fuscous, initially phragmosporous, later paucimuriform (15.8–)18.5(–21) × 26.5–39.5(–60) μ, often with a conspicuous, disjunctiformed hilum.

Material examined: FH, Rabenhorst, Fung. Europ. Exsic. 985, SYNTYPE, on dead *Lycium europaei* branches (slide RTM 1:154).

Steganosporium Celtidis (Syd.) comb. nov. FIG. 16.
≡ *Sporodesmium Celtidis* P. Sydow, Hedwigia Beibl. **38**: 144. 1899.

Acervuli borne within orbicular patches 2–4 mm in diameter; conidia oval to oblong, 5–15-celled, fuscous, 21–28.5 × 12.5–16 μ, borne on hyaline, single-celled, stout conidiophores that upon disjunction leave a conspicuous scar.

Material examined: S (Sydow coll.), TYPE, Herzegovina, Mostar, Yugoslavia, on *Celtis australis* (slide RTM 1:101).

Steganosporium foliicolum (Desm.) comb. nov. FIG. 15.
≡ *Sporidesmium foliicolum* Desmazières, Ann. Sci. Nat. II **14**: 8–9. 1840.

Acervuli initially punctiform, conspicuously superior to the host tissue, upon aging becoming flattened, and if close together becoming confluent; in the material examined, and as noted by Desmazières, only the lower epidermis being parasitized and then the fungus appearing to attack only the cuticle and not to penetrate the epidermal cells; conidia sessile, basally terminating in a pedicel, essentially oblong, though tending to be irregular, multicellular, somewhat spumescent, thin-walled, pale fuscous, 10.5–23.5(avg. 15.8) × (5–)8(–10.5) μ.

Material examined: NY (Ellis coll.), PC (authentic), 1083 (1st ed.) and 483 (2nd ed.) resp., on *Quercus leaves* (slides RTM 1:81, 1:113).

Hughes (**10**) places this species (cited as *Sp. foliicola*) in synonomy with *Stigmella dryophylla*. However, it neither penetrates the epidermis nor produces pycnidia, but rather appears to develop only between the epidermis and cuticle. Upon maturation it ruptures the cuticle and appears as a typical acervulus. For these reasons it is placed among the *Steganosporia*.

The four species of *Steganosporium* represented here would suggest that perhaps the genus is divisible into two parts. The portion represented by *pyriforme*, *Lycii* and *Celtidis* is characterized by conidia that are regularly septate and initially phragmosporous, the spores becoming muriform as they mature or age, but sometimes remaining transeptate, and produced as blownout ends of the conidiophores, which tends to give to them a uniformity of width and a regularity of outline. This mode of production and disjunction leaves a distinctive basal scar. The conidia of *S. foliicolum*, on the other hand, are spumescent as though

the developing spore was produced by random blowouts of the cell wall, which gives to them an irregular form, reminiscent of foam. The spore in this case is an integral extension of the conidiophore and when it breaks away it not infrequently carries with it a short stem. Only a definitive study of the *Steganosporia* will determine whether the *foliicolum* type is unique or represents a more general ontogenetic pattern that should, perhaps, form the basis of a separate genus.

THE SPORODOCHIAL ANALOGUE

Initial searches in this segment of the complex lead me to believe that perhaps these species could be placed in *Coniosporium* as interpreted by Mason (**13**) in 1933 and later by Hughes (**9**) in 1951. However, examination of slides (in Herb. QM) prepared by Damon from type material (ex Herb. B) of the type species, *C. olivaceum*, shows the conidia to be catenate (FIG. 6). Inasmuch as *Coniosporium* was compiled by Fries in 1832, Damon in his notes (in Herb. QM) suggests that this may be an earlier name for *Sirodesmium* described by de Notaris in 1849. Quite accidentally "*Berkleasmium* Zobel = *Sporodesmium* (Monil.), fide Sacc." was encountered in "A Dictionary of the Fungi." Zobel based his new genus on *Sporidesmium concinnum* Berkeley, but in memory of Corda illegitimately coined the name *B. Cordeanum*. Nevertheless, examination of authentic material has convinced me that this long ignored monotypic genus provides the correct name for the sporodochial members of our complex.

BERKLEASMIUM Zobel

Colonies sporodochia; conidia phaeodictyous, borne on short, simple conidiophores, or else sessile.

TYPE: *Sporidesmium concinnum* Berk.

Berkleasmium concinnum (Berk.) comb. nov. FIGS. 9, 10.

≡ *Sporidesmium concinnum* Berkeley, Hooker's Lond. Jour. Bot. **4**: 309. 1845.

≡ *Berkleasmium Cordeanum* Zobel, in Corda, Icones Fung. **6**: 4. 1854.

Sporodochia saprobic, black, distinct, about 0.2 mm in diameter, compact, where crowded tending to become confluent; conidia broad-cylindrical, multicellular, cells large and fairly regular, fuscous, borne on short conidiophores which become obscure with maturity, 60–105 × 23.5–30 μ.

Material examined: FH (Curtis coll.) (authentic), on dead wood (slide RTM I:20).

Berkleasmium granulosum (Dur. & Mont.) comb. nov. FIG. 12.
≡ *Sporidesmium granulosum* Durieu & Montagne, in Durieu, Flore d'Algérie, Cryptogamie, p. 330. 1847.

Sporodochia on dead wood, distinct, punctiform; conidia subglobose, to oval to irregular cylindrical, sub-fuscous, composed of irregular cells irregularly ordered, borne on short pedicels which may or may not be separated from the spore by a septum, quite variable in size, 29–66 × 16.5–18.5(–35) μ, but with length always exceeding breadth. The sporodochia when treated with weak KOH yield a rose-biege solution.

Material examined: PC (Durieu coll.), TYPE, Philippeville, Algeria, on old *Olea* wood (slide RTM I:115).

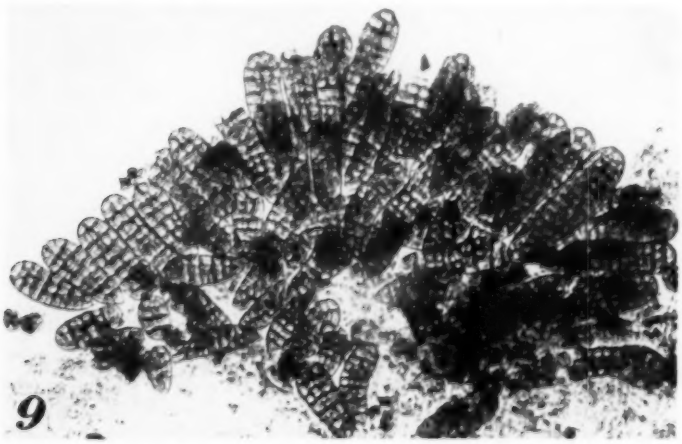


FIG. 9. *Berkleasmium concinnum*, *sporodochia*, × 225.

Beakleasmium conglobatum (Cke. & El.) comb. nov. FIGS. 2, 11.
≡ *Sporidesmium conglobatum* Cooke & Ellis, Grevillea 8: 11. 1879.

Sporodochia distinct, black, the crowded conidia giving to them a corrugated appearance; conidia multicellular, cells small and quite regular, deep fuscous to opaque, oval to ovate, sometimes terminating in a short apiculus, 26–47 × 36.5–99 μ; borne on short, stem-like conidiophores that may or may not be separated from the spore by a septum.

Also present on the type material are ascocarps of *Hysterographium Mori (Rousselii)*, but since Lohman (**15**) has reported this species to have a pycnidial phase they are probably not associated with *B. conglobatum* (note also Bisby (**1**)).

Material examined: NY (Ellis coll. 3172), TYPE, Newfield, N. J., on old wood (slide RTM 1:30).

THE DEMATIACEOUS ANALOGUE

This portion of the complex comprises most of the species. The genus *Sporidesmium* was established by Link in 1825 for his *S. atrum*. Fries, 1832, compiled the genus and referred to the conidia as being "transverse septatis atris"—*i.e.*, phaeophragmosporous. However, he added five additional species of his own, and of these, one, *S. cellulosum*, was described as having conidia that were "longitudinaliter cellulosis et transversaliter septatis, atris"—*i.e.*, phaeodictyosporous. In 1849 in the *Summa Vegetabilium Scandinaviae* Fries attempted to rectify this prime source of confusion by restricting *Sporidesmium* to those species with "Sporidia fasciculata, stricta, subclavata, transversim septata, stipite brevi aut nullo." Although in a footnote he suggested disposition for certain *Sporidesmia*, he made no new combinations and left the genus in its original heterogenous state. In the same year that Fries compiled *Sporidesmium* Schweinitz in the *Synopsis fungorum in America Boreali degentium* erected *Clasterisporium* with the single species *C. caricinum* (FIG. 4). This fungus is specifically distinct on the basis of producing hyphopodia, but on the basis of the conidia and their production the genus can only be synonymous with *Sporidesmium*, as typified by *S. atrum*. The Ellises (**6**) have recently thoroughly described and figured living material of *C. caricinum* and their description is in perfect accord with type material examined ex Herb. PH (Schweinitz coll. 2998) (slide RTM 1:71). Also, in 1839, Corda established *Hymenopodium* for the single species *H. sarcopodioides*. Examination of the type specimen (PR 515138, Corda coll. (slide RTM 1:137)) confirms Hughes's (**8**) examination of the same material in which he ascertained it to be conspecific with *C. caricinum*. A final genus that is implicated is *Stigmella* which Saccardo in 1878 included in the Hyphomycetes, and the species ascribed to it merge indistinguishably with *Sporidesmium*. Hughes (**10**), however, has presented presumptive evidence that the type, *St. dryophylla* (Corda) Lindau, is pycnidial. In 1882 Saccardo made a now invalid attempt to adjudge this taxonomic disorder by emending *Sporidesmium* to include only Dictyosporae, making *S. cellu-*

losum the type of his emended genus, and transferring the phragmosporous species to *Clasterisporium*.

The foregoing summary necessitates the following synonymy and transfer:

SPORIDESMIUM Link ex Fries
= *Clasterisporium* Schweinitz, Am. Phil. Soc. Tr. II **4**: 300. 1832.
= *Hymenopodium* Corda, Icon. Fung. **1**: 7. 1837.

Colonies hyphomycetous, sparse; conidia phaeophragmous, borne monacrogenously on short simple conidiophores, or sessile.

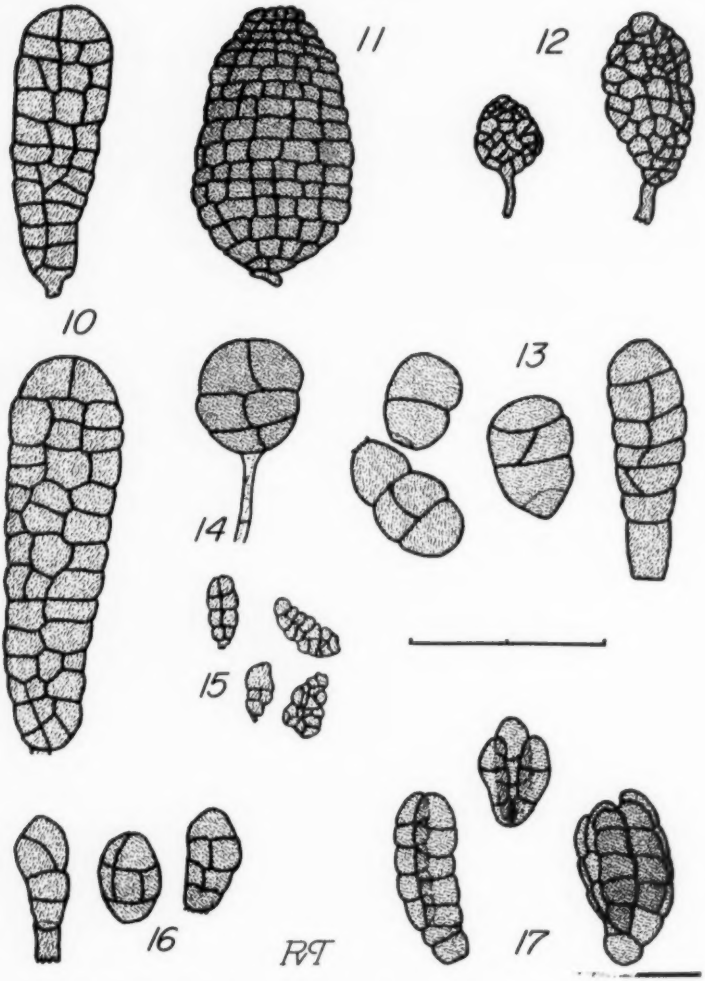
TYPE: *Sporidesmium atrum* Link ex Fries.

Sporidesmium caricinum (Schw.) comb. nov. FIG. 4.
≡ *Clasterisporium caricinum* Schweinitz, Am. Phil. Soc. Tr. II **4**: 300. 1832.
≡ *Sporidesmium clasterisporium* (Schw.) Corda, Icones Fung. **6**: 4. 1854.
= *Hymenopodium sarcopodioides* Corda, Icones Fung. **1**: 7. 1839.
= *Sporidesmium sarcopodioides* Bonorden, Handb., p. 48. 1851.
= *Clasterosporium* [!] *sarcopodioides* (Corda) Saccardo, Michelia **2**: 22. 1880.

In 1914 Bubák (**2**), recognizing that the type of *Stigmella* was pycnidial, erected the genus *Piricauda* for *Stigmella Ulcana* Sacc. Although with specifically unique, long, tapering conidiophores, its general morphology coincides with the already existing generic definition of *Sporidesmium* and *Stigmella* sensu Saccardo. *Piricauda* by virtue of being ignored has been given the not inconsiderable advantage of remaining monotypic, thereby obviating the need for further taxonomic studies in this genus.

PIRICAUDA Bubák emend.
≡ *Stigmella* sensu Saccardo, Michelia **1**: 264. 1878.
≡ *Sporodesmium* sensu Saccardo, Michelia **2**: 23. 1882.

Colonies micronemic, at maturity sometimes consisting of a crust of crowded conidia; conidiophores simple, unbranched, composed of several cells, or else reduced to a single cell or peg, generally arising directly from the substrate; conidia phaeodictyous, monacrogenous, echinulate or smooth, ranging in shape from sphaerical to elliptical to cylindrical to obspathulate to obovate to pyriform, pauci- (less than 10) to multilocular with cells regular or irregular in shape, and with thin or thick walls; dis-



FIGS. 10–12. *Berkleasmium*. 10. *B. concinnum*. 11. *B. conglobatum*. 12. *B. granulosum*. FIGS. 13, 15, 16. *Steganosporium*. 13. *S. Lycii*. 15. *S. foliicolum*. 16. *S. celtidis*. FIG. 14. *Piracauda paraguayense, Ule 948*. FIG. 17. *Cheiromyces montellica*, conidia from Bubák material. (Scale line equals 50 μ.)

rupted sessile spores frequently subtended by a torn remnant of fertile hyphae.

Type: *Stigmella Uleana* Saccardo & Sydow.

Piricauda paraguayense (Speg.) comb. nov. FIGS. 1, 3, 14.

≡ *Stemphylium paraguayense* Spegazzini, An. Soc. Cient. Argentina **22**: 217. 1886.

= *Sporidesmium Durantae* Patouillard, Bull. Herb. Boissier **3**: 74. 1895.

= *Stigmella scitula* Sydow, in Theissen, Ann. Myc. **7**: 343. 1909.

= *Stigmella Uleana* Saccardo et Sydow, in Saccardo, Rend. Cong. bot. Palermo, Maggio, pp. 12–13. 1902.

= *Piricauda Uleana* (Sacc. et Syd.) Bubák, Ann. Myc. **12**: 218. 1914.

Colonies saprobic, appearing araneose from the conidiophores; conidia globose to napiform and turbinate (pyriform), deep fuscous to opaque, paucicellular, becoming thick walled, 18.5–24 × 24–31.5 μ; conidiophores pale fuscous to subhyaline, long-flexuous, 50–90 μ high, tapering from about 5 μ thick at the distal end to about 2.5 μ thick at the base, several-celled, arising directly from the substrate.

Material examined: LPS 13001 (Spegazzini coll. 3837), TYPE, Peribebuy, Paraguay (slide RTM I:99). BKL (Bubák coll.): Brazil, on *Citharexylon, Ule 948*, TYPE of *P. Uleana* (slide RTM I:18); São Leopoldo, Brazil, on *Bignonia*, leg. *Theissen* (slide RTM I:19). S (Sydow coll.), São Leopoldo, Rio Grande do Sul, Brazil, on *Bignonia* leaves, leg. *Theissen* (slide RTM I:102). FH (Patouillard coll.), Pululahua Crater, Ecuador, on *Duranta* leaves, leg. *Lagerheim*, TYPE of *Sp. Durantae* (slide RTM I:202).

SYNOPSIS OF CORRECT MONACROGENOUS GENERA TREATED IN TEXT

I. Conidia furcate ..... *Cheiromyces* / *Dictyosporium*

II. Conidia phaeophragmous, borne on short, simple conidiophores; colonies of the dematicaceous type ..... *Sporidesmium*

III. Conidia phaeodictyous

A. Conidia borne in pycnidia (Phomaceae) ..... *Stigmella*

B. Conidia borne in acervuli (Melanconiaceae) ..... *Steganosporium*

C. Conidia produced on sporodochia (Tuberculariaceae) ..... *Berkleasmium*

D. Conidia produced from colonies of the dematiaceous type

1. Conidia produced as protoplasmic extensions of the conidiophore, but never with any continuity of wall between conidium and conidiophore ..... *Stemphylium*

2. Conidia as modified hyphal termini, borne on teeth, conidiophores, or sessile ..... *Piricauda*

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I wish to extend my sincere appreciation to Dr. H. L. Barnett for recent material of *Steganosporium pyriforme*, to Dr. M. L. Lohman for his sagacious examination of our exsiccatum of the same species, and to Dr. E. G. Simmons for his discernment of *Stemphylium*. This study was initiated at the Quartermaster Research and Development Center, but elaborated and brought to culmination under the direction of Dr. I. Mackenzie Lamb, Director of the Farlow Herbarium.

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THE SAPROLEGNIACEAE OF FLORIDA [1]

A. W. Ziegler

A state-wide survey of the Saprolegniaceae of Florida has never been made. The few records of systematic collections made within the state (**5**, **6**) yield no information on the species which are prevalent. Florida is well suited for a study of water molds because of its abundant lakes, swamps, streams, drainage ditches, and canals. The author has made extensive collections in all areas of the state and has identified the water molds isolated from the collections.

COLLECTING

The methods of collecting and isolation were the familiar and standard ones described by Johnson (**3**). At the beginning of the study, soil and water samples were collected in one-day trips to nearby counties. A water and soil sample was taken from each collecting site. Most of the collections from central and south Florida were obtained during the early summer of 1956. These samples were sent to the mycology laboratory of Florida State University at Tallahassee by air express. The time lapse between collections and the beginning of processing in the laboratory seldom exceeded 24 hours. A return trip to the southern areas was made during November 1956 to "spot collect" during cooler weather.

In certain areas of the state, such as Liberty, Osceola, and Putnam counties, collecting sites were so abundant that a selection could be made. In other areas, such as Suwannee and Marion counties, collecting areas were difficult to find because of a prolonged drought, although enough sites were finally located to survey the counties adequately. In general, soil and water samples taken from the many lakes of central Florida, the canals of south Florida, and the restricted fresh-water sources on the Florida Keys failed to yield many water molds. Inland areas of central and south Florida, south to the Tamiami Trail, provided an abundance of excellent collection sites.

[1] The author is indebted to the National Science Foundation for grant #2013 which made this work possible. He is also indebted to Dr. Leland Shanor and Dr. T. W. Johnson for their reading and criticism of the manuscript and to Mr. Gilbert Hughes for his aid in collecting and identification and the use of portions of his master's thesis.

Ideally, two collections should have been made at a specific site, one from each temperature extreme. The basis for this statement is explained in the last section of the paper. Because of distances in the state, however, this was not found to be feasible. In south Florida, it is doubtful whether summer and winter water temperatures would be vastly different.

RESULTS

In all, 1615 water and soil samples were obtained from the 67 counties of Florida. From these, 1285 isolates were cultured and identified. Ninety-four of the isolates were representatives of families other than the Saprolegniaceae.

To conserve space, a listing of the species identified together with the number of collections of each and very brief remarks on distribution is included, rather than a complete listing by counties and ecological data on the collection areas.

SEASONAL OCCURRENCE

For several years, the author has noted that certain water fungi can be isolated from soil and water samples only during hot or cold weather, while others appear at all seasons. In general, for this climate, it can be said that saprolegniaceous fungi with eccentric oospores tend to dominate collections made during warm weather, while those with centric and subcentric oospores rarely occur in samples made during warm weather but are of frequent occurrence in cooler weather.

Although it would have been possible to record the temperatures of the samples at the time of collecting, it would not have been feasible to maintain these samples at the recorded temperatures during incubation. When a grouping by month of the samples is made, however, it is evident that the eccentric-oospore species, constituting about 75% of the total water molds identified, occur equally in cool and hot weather, whereas the centric- and subcentric-oospore species, constituting 25% of the total, occurred mainly (80%) in cooler weather.

An approach to an hypothesis of the influence of temperature on occurrence of water molds was shown by Coker (**1**, p. 14) in his "Periodicity Table." These data indicate that certain species may be of seasonal occurrence. As he stated, though, "if we compare the six cold and the six warm months we find little or no difference, ten (species) appearing more frequently in the warm and nine (species) in the cold weather."

SAPROLEGNIACEAE IDENTIFIED

Species	No. of collections	Remarks
Achlya		
americana	42	Widely distr.; mostly hot weather.
apiculata	5	Cool weather; N. Fla.; new record.
caroliniana	11	All seasons; new record.
colorata	26	Cool weather; wide distr.; new record.
conspicua	33	Cold weather; wide distr.
crenulata	3	S. & C. Fla; new record.
dubia	24	Hot weather; wide distr.; new record.
flagellata	254	All counties; all seasons.
hypogyna	6	Cold weather; wide distr.; new record.
klebsiana	4	Hot weather; new record.
lobata	4	All seasons; new record.
megasperma	2	Cool weather; N. Fla.; new record.
mucronata	2	N. Fla.; cool weather; new record.
oblongata	10	Hot weather; C. & S. Fla.; new record.
orion	12	All seasons; wide distr ; new record
prolifera	36	All seasons; wide distr.
proliferoides	80	All seasons; wide distr.
racemosa	15	All seasons; N. Fla.; new record.
recurva	7	Hot weather; wide distr.; new record.
rodriqueziana	5	Hot weather.
treleaseana	29	Cool weather; wide distr.; new record.
Aphanomyces		
laevis	13	All seasons; wide distr.
scaber	9	All seasons; wide distr.; new record.
stellatus	3	All seasons; new record.
sp.	2	Not producing oogonia.
Brevilegnia		
diclina	4	Hot weather; wide distr.; new record.
globosa	1	New species.
linearis	10	Hot weather; C. & S. Fla.; new record.
unisperma	11	Hot weather; new record.
Dictyuchus		
missouriensis	25	All seasons; wide distr.; new record.
monosporus	244	All seasons; all counties.
pseudodictyon	56	All seasons; wide distr.
Geolegnia		
inflata	3	New record.
Isoachlya		
eccentrica	2	Cool weather; N. Fla.; new record.
intermedia	7	Cool weather; new record.
toruloides	3	Cool weather; N. Fla.; new record.
unispora	9	Cool weather; N. Fla.; new record.
Leptolegnia		
caudata	9	Cold weather, N. Fla.; new record.
sp.	1	Cold weather; no oogonia formed.
Thraustotheca		
clavata	10	Hot weather; N. & C. Fla.; new record.
primoachlya	16	All seasons; mostly N. Fla.; new record.
Saprolegnia		
delica	25	Cold weather; wide distr.
diclina	37	Cold weather; wide distr.; new record.
ferax	50	Cold weather; wide distr.
litoralis	22	Cold weather; wide distr.; new record.
megasperma	1	Cold weather; S. Fla.; new record.
monoica var. *glom.*	8	Cold weather; N. Fla.; new record.

In 1956–1957, Hughes (**2**) made 500 collections of water and soil samples from an area of south Georgia. One-half were made in September with a water and soil temperature range from 17–37° C, and the remainder in winter with a range from 7–22°. In the September samples, eccentric-oospore fungi were dominant and only 4% subcentric- or centric-oospore water molds of the totals were isolated. In the winter collections, however, 55% of the isolates were water molds with centric or subcentric oospores.

In her paper on the water molds of Puerto Rico (**4**), a country with a fairly constant warm temperature, Miss Rossy-Valderrama reported only one subcentric-oospore species. All others were those with eccentric oospores.

While it is not possible with the data at hand to prove conclusively that in this climate there is a definite variation in occurrence, the data do indicate that such a variation may exist. A study of the factors which might affect a seasonal variation is now being made.

SUMMARY

1. During a survey of the water molds of the state of Florida, 1615 soil and water samples were collected. From these samples 1285 fungi were isolated and identified. Of this total, 94 isolates were not members of the family. A list of the fungi isolated with the number of times each species was collected and brief remarks on each is included.

2. Some evidence not yet conclusive is presented for seasonal variation in species occurrence in this climate.

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COCHLIOBOLUS SATIVUS. I. PERITHECIAL DEVELOPMENT AND THE INHERITANCE OF SPORE COLOR AND MATING TYPE[1]

R. D. Tinline and J. G. Dickson

(with 5 figures)

Perithecia of *Cochliobolus sativus* (Ito & Kurib.) Drechs. ex Dastur, the perfect state of *Helminthosporium sativum* P. K. & B. (*H. sorokinianum* Sacc. ex Sorokin), have not been reported in nature. They have been obtained, however, in laboratory cultures (**3**, **4**, **8**, **9**). Tinline demonstrated that the fungus was heterothallic, each thallus being bisexual and self-sterile. He designated two mating types as *A* and *a* (**9**). Shoemaker (**8**) and Hrushovetz (**3**) studied the cytology of the developing ascocarp. The former author also reported on the nature of sexuality in the fungus. He found that uninucleate, spherical spermatia were the male agents in plasmogamy; ascogonia were the female reproductive organs.

The characteristics of heterothallism and sexuality indicated that the perfect stage might be used as a tool to study genetically the variation in the organism. Abundant material was required for such an investigation. Although culture methods for perithecial formation were described by Tinline (**9**) and after by Shoemaker (**8**), a determination of the environmental conditions that were favorable for the development of the sexual stage appeared necessary. A knowledge of favorable environmental conditions also may be of practical importance in that it may assist in a search for the perfect stage in nature. The purpose of the present paper is to report on the effects of some environmental factors on the formation and maturation of perithecia, and, in addition, on the inheritance of spore color and mating type.

[1] Joint contribution from the Botany and Plant Pathology Division, Science Service, Department of Agriculture, Ottawa, Ontario, Contribution No. 1677, and the Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.

Most of the work reported here formed part of a thesis presented by the senior author to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for a Ph.D. degree, June 1954.

MATERIALS AND METHODS

Isolates of *C. sativus* were maintained on test-tube slants of potato dextrose agar at approximately 22° C. The sexual stage was promoted by growing compatible isolates intermixed on Montcalm barley kernels which were placed on Sachs's nutrient agar (SNA) in Petri dishes after the method of Tinline (**9**). The composition of the SNA in grams per liter of distilled water was as follows: $Ca(NO_3)_2$, 1.0 g; KCl, 0.25 g; K_2HPO_4, 0.25 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; $FeCl_3$ or $FeC_6H_5O_7 \cdot 3H_2O$, trace; $CaCO_3$, 3.0 to 4.0 g; and agar, 20.0 g. The initial pH of the medium was 6.0, approximately. Prior to plating, the barley grains were soaked for 1 to 4 hours in water, surface-sterilized with mercuric chloride (1:1000), washed with sterile distilled water, and boiled in water for a minute or two to kill the embryos. They were inoculated by immersion in an aqueous suspension of conidia from two isolate cultures.

The plates were wrapped in paper to eliminate light in all tests where light was not a considered factor. Except in the temperature tests, the cultures were incubated for seven days at 24° and for 14 days at 20°. The hydrogen-ion concentration of the media was adjusted to and maintained at desired levels by addition of phosphoric acid or its potassium salts or both (**10**). The final concentration of the phosphate buffers was 0.05 molar.

Two monoascosporic isolates, a white-spored strain of mating type *A* and a wild-type olivaceous-spored strain of mating type *a*, were employed throughout the environmental study. The white-spored strain was derived from a cross between a white fertile saltant which was obtained by ultraviolet radiation (**2**) and a dark-spored strain. Occasionally other isolates were included to increase the replication within the tests.

The results of the environmental tests were determined in terms of the relative numbers of perithecia (proto- and pseudothecia), asci (in pseudothecia), and ascospores (in mature pseudothecia) that developed in the cultures.

In the genetic study segregation ratios were obtained from random spore analysis and ascus (tetrad) analysis (**1, 7**). The method used to isolate all the spores of an ascus was as follows: Perithecia that were freed of debris and adhering spores by rolling them across an agar surface were crushed in a water droplet; if examination showed abundant ascospores the material was washed from the slide to an agar surface in a Petri dish; an ascus containing spores was selected and moved apart

from the perithecial material; the ascus wall was ruptured with sharp steel needles; the spores were withdrawn from the ascus case, uncoiled, and distributed singly on a free agar surface in the dish; after spore germination (4 to 12 hours) blocks of agar that contained individual spores were transferred to fresh medium in Petri dishes or test tubes.

The presence or absence of pseudothecia was the criterion for mating-type reaction. The mating type to which an isolate belonged was determined by growing the isolate singly, and together with each of two tester isolates of opposite reaction and of a spore color unlike that of the isolate under test.

RESULTS

ENVIRONMENTAL FACTORS

The effect of two variations of constant temperatures on the formation of perithecia were investigated. Firstly, cultures were incubated for seven days at room temperature, then for 14 days at the different constant temperatures. Secondly, cultures were incubated at the different constant temperatures for 21 days.

TABLE 1*

EFFECT OF TEMPERATURES ON THE DEVELOPMENT OF COCHLIOBOLUS SATIVUS

Constant temperature °C	Incubation time—7 days at room temperature					
	14 days at constant temperature			21 days at constant temperature		
	Perithecia	Asci	Ascospores	Perithecia	Asci	Ascospores
8	+					
12	++					
16	+++	++	+			
20	+++	++	++	+++	++	+
24	++	+		++	+	
28	+					

* The symbols +++, ++, etc. indicate relative numbers of perithecia, asci, or ascospores.

The data of TABLE 1 show that mature perithecia were formed more abundantly at 20° than at higher or lower temperatures, regardless of the temperature of incubation for the first seven days. Cultures held at room temperature for a week, however, produced perithecia over a wider range of temperatures than cultures incubated at constant temperatures for 21 days.

The results of numerous tests indicated that a temperature of 24° was equal to or better than room temperature during the first week of

incubation in influencing perithecial formation. Controlled temperatures were preferred in the experimental work. Consequently 24° was taken as the optimum temperature for the first seven days.

Perithecia developed over a wide range of alternating temperatures (TABLE II). The cultures were alternated every 12 hours between two constant temperatures during the 21 day period.

TABLE II*

EFFECT OF ALTERNATING TEMPERATURES ON THE DEVELOPMENT OF COCHLIOBOLUS SATIVUS

Temperature °C	Perithecia						Asci						Ascospores					
	12	16	20	24	28	32	12	16	20	24	28	32	12	16	20	24	28	32
12		++	+++	+++	++	+			+	++	+							
16		++	+++	+++	++	++		+	+++	+++	++					++		
20			+++	+++	+++	++			+++	+++	++	+			++	++		
24				+++	+++	++				+++	+					++		
28					+													
32																		

* Symbols defined in TABLE I.

Mature perithecia were more numerous in cultures incubated in the dark than in cultures exposed to sunlight. Further, they occurred at a greater number of different temperatures under the former than the latter light condition (TABLE III).

TABLE III*

EFFECT OF LIGHT ON THE DEVELOPMENT OF COCHLIOBOLUS SATIVUS

Temperature °C	Exposed to light			Unexposed		
	Perithecia	Asci	Ascospores	Perithecia	Asci	Ascospores
16	+	+		+++	+++	++
20	++	+		+++	+++	++
24	++	++	+	+++	+++	++
28	+			+	++	

* Symbols defined in TABLE I.

The function of an agar medium was found to be non-specific. Perithecia were formed on seed that was placed on SNA, water agar, malt agar, and corn-meal agar. Approximately equal numbers of mature perithecia developed on seed that was placed on SNA and on water agar, each of initial pH 6.0. Profuse asexual growth of the fungus occurred on malt and corn-meal agars. This hindered the rapid detection of perithecia in the cultures.

Perithecia did not develop on agar media when plant parts such as cereal grains were omitted as a substrate. Further, a cold sterilized homogenate from barley seed that was incorporated in water agar and buffered at various pH levels did not support the formation of the perfect stage.

The hydrogen ion concentration of agar media was a critical factor in perithecial production. The pH of barley seed and that of the water agar were adjusted to various levels. The procedure employed to change the pH of the seed was as follows: samples of seed were soaked for eight hours in water buffered at various pH levels; then surface-sterilized with an unbuffered solution of mercuric chloride (pH 6.0); next, washed in several changes of water buffered at the same pH as the water in which the seed was soaked; and finally, boiled in the buffered water to kill the embryos. The pH of the water in which seed was soaked and the initial pH of seed determined after homogenization with distilled water in a Waring blender were 3.6 and 5.1, 6.0 and 5.4, 6.2 and 5.6, 8.0 and 6.3, and 11.4 and 7.5, respectively. All combinations of pH were set up between the seed and agar media.

The pH of the seed tended to parallel that of the agar medium on which it was placed. Further, perithecial development was similar on an agar medium regardless of the pH of the seed. Consequently, only the range in final pH of the agar media and the seed is presented in TABLE IV.

TABLE IV*

EFFECT OF pH ON PERITHECIAL DEVELOPMENT IN COCHLIOBOLUS SATIVUS

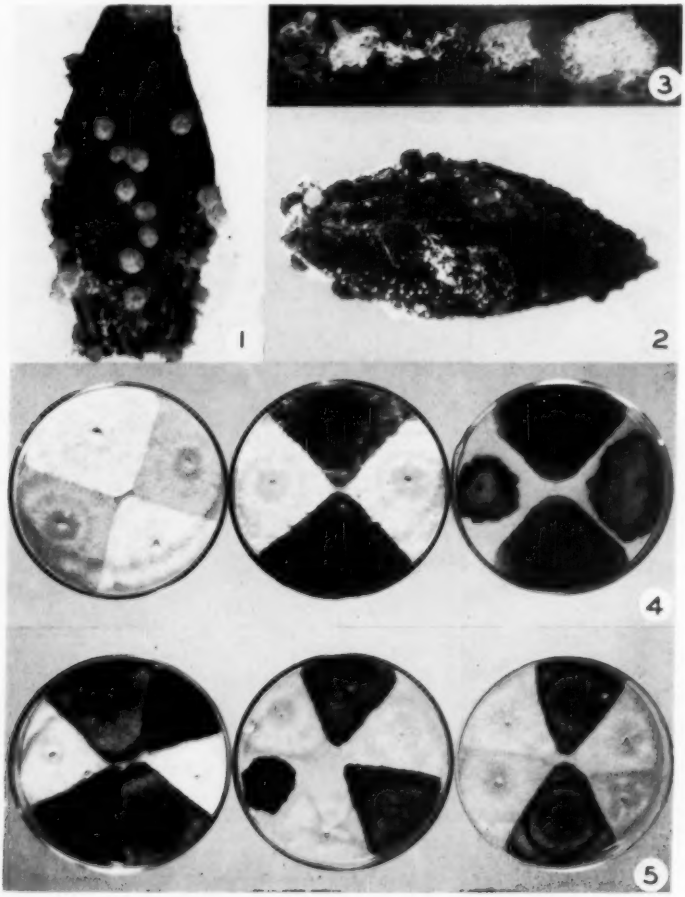
Water agar		Barley seed Final pH†	Perithecia	Asci	Ascospores
Initial pH	Final pH†				
4.0	4.8–5.1	5.4–5.7	+ + + + +	+ + + +	+ + +
5.8	5.8–6.1	6.0–6.3	+ + +	+ + +	+ +
6.0	6.2–6.6	6.3–6.9	+ + +	+ + +	+ +
7.5	7.2–7.5	6.8–7.5	+ +	+	
9.1	8.5–8.6	7.0–7.8	+		

* Symbols defined in TABLE I.
† pH of water homogenate of substrate and fungus.

The formation and maturation of perithecia were favored by an acid reaction and suppressed by an alkaline reaction of the agar media (TABLE IV). No differences in vegetative growth were observed between the cultures. In additional tests of pH, perithecia formed in cultures on media of pH 6.0 but not on media of pH 3.5 or 7.7.

GENETICS

The bisexual self-sterile nature of isolates of *C. sativus* was confirmed by cultural methods. Black and white perithecia developed in cultures of compatible white and olivaceous strains (FIGS. 1–3). Evidently reciprocal fertilization occurred. Ascospores that were isolated



FIGS. 1–5. *Cochliobolus sativus*. 1–3. Black and white perithecia. 1–2. Perithecia *in situ* on barley kernels, × 10. 3. Perithecia enlarged, × 36. 4–5. Ascus analysis for conidial color. 4. Four-spored asci. 5. Six-spored asci.

from black or white perithecia or from an ascus from such perithecia produced white- and dark-spored colonies.

Apparently spore color is controlled by an allelic gene pair. A total of 287 ascospores was isolated non-selectively from white and black perithecia which were formed in cultures of various pairs of isolates. The conidial color of monoascosporic colonies was visible about four days after isolation of the spores. The ratio of 145 white to 142 olivaceous colonies that was obtained on the basis of conidial color closely approached the expected 1:1 ratio. Further, without exception, the spores from eight-spored asci produced four white and four olivaceous colonies. All the possible combinations of color under the 1:1 segregation ratio occurred when the number of spores per ascus was less than eight. Examples of the segregation from four- and six-spored asci are illustrated in FIGS. 4 and 5, respectively.

The mating types *A* and *a* appeared allelic. Of fifty-six monoascosporic isolates which were selected randomly, twenty-six belonged to mating type *A* and thirty to mating type *a*.

TABLE V

RANDOM-SPORE ANALYSIS FOR MATING TYPE AND SPORE COLOR IN COCHLIOBOLUS SATIVUS

Spore color	Mating type		Total
	A	a	
W	16	13*	29
w	10*	17	27
Total	26	30	56

* Parental genotype.

Spore colors—i.e., white (*w*) and olivaceous (*W*)—and mating types—i.e., *A* and *a*—were inherited independently. The data of TABLE V show that the recombination frequency exceeded 50 per cent of the population. Further, the difference between the observed and expected numbers of isolates falling into the four genotypic classes was not significant by chi-square test.

Tetrad analysis failed to confirm the independence of the two characters which was established by random-spore analysis. The classification of tetrads from 29 analyses was as follows: 23 tetratypes; 2 parental ditypes; and 4 non-parental ditypes. The frequency of the first class compared with those of the two remaining classes made further analysis for spore color and mating type unwarranted. In addition, some mem-

bers of some tetrads reacted with the tester isolates in a manner not previously encountered in this fungus.

Apparently some monoascosporic isolates exhibited intra- as well as inter-group fertility; that is, they reacted with each of two tester isolates which were of unlike mating type. The intra-group reactions occurred most frequently with the white-spored and rarely with the dark-spored tester strains. Homothallic isolates were not found. Possibly additional factors other than the mating-type factors *A* and *a* conditioned heterothallism in the fungus.

DISCUSSION

Some environmental conditions influenced the formation of mature perithecia of *C. sativus* in laboratory cultures. Substrate, temperature, and pH appeared to be of prime importance. The optimum combination of temperatures for maturation of perithecia within 21 days was 24° for 7 days followed by 20° for 14 days. Possibly under longer periods of incubation, combinations of temperatures below 20° may be optimum. Shoemaker subsequently confirmed and, through his discovery of the nature of sexuality in the fungus, explained the necessity of the two temperatures of incubation (**8**). He found that a temperature of 24° matured spermagonia when ascogonia were first formed in the cultures; hence spermatization was facilitated. Ascosporogenesis, however, was favored by a temperature of 20°.

The sexual stage of the fungus required more precise environmental conditions for development than the conidial stage. Vegetatively the fungus grew between 4 and 36° with optimum at 28°; between pH 3 and 12 with optimum at pH 8; and on simple synthetic as well as natural media (unpublished data). Sexually the fungus developed between 16 and 24° with optimum at 20°; between pH 4 and 6, optimum not determined; and on some natural media only. Since the same strains were used throughout, the differences between the sexual and vegetative stages in temperature and pH optima were probably not due entirely to substrate differences. Apparently some conditions that suppressed asexual growth favored perithecial development.

The apparent absence of perithecia in the field cannot be adequately explained by the results obtained in the pure-culture experiments. Although light appeared detrimental to the delimitation of ascospores, perithecia were formed under most light conditions. The temperature and hydrogen-ion concentration that were conducive to perithecial formation in laboratory cultures may be met with in nature. Culms, wheat heads, and various cereal grains supported the production of the perfect

stage (**8**, **9**). Hermaphroditic strains of opposite mating types were reported to occur in nature (**9**). Presumably, combinations of environmental factors favorable for perithecial development occur under natural conditions. The total absence of records of field collections of perithecia of *C. sativus* would thus suggest either that the combinations of factors prerequisite to perithecial formation occur very infrequently, or that in the field there are other factors inimicable to perithecial production. In the latter connection it is known that *C. sativus* is sensitive to the products of other micro-organisms. Possibly competition or antibiosis or both may inhibit the formation of perithecia under natural conditions.

In this and in previous work numerous isolates were tested for mating-type reaction and were readily classified as *A* or *a*. The sudden occurrence of isolates which appeared intra-group fertile and hence difficult to classify requires investigation. Such isolates were progeny from a cross between an irradiated strain or spontaneous mutant and an untreated strain. One explanation therefore may be that a mutation in mating type occurred and was transmitted to some of the progeny. An alternate explanation may be that mutation in mating type or contamination with a strain of opposite reaction occurred in one tester strain of each spore color. The phenomenon of intra-group fertility, however, is not new in the genus *Cochliobolus*. Nelson reported such a phenomenon in *C. heterostrophus*, the perfect stage of *Helminthosporium maydis*, and suggested that at least two factors other than compatibility factors *A* and *a* governed the production of mature ascocarps (**5**, **6**). Since he worked with monoconidial isolates obtained from naturally diseased corn leaves, the possibility of intra-group fertile isolates of *C. sativus* from field infections should not be overlooked. Further studies on the genetic basis of heterothallism in *C. sativus* are in progress.

SUMMARY

The formation and maturation of the sexual stage of *Cochliobolus sativus* in laboratory cultures were greatly affected by environmental factors. Perithecia failed to develop on media from which plant parts were excluded. The agar media served only as moisture sources; the nutritive elements in the media were non-specific in function except as buffers to maintain a pH conducive to perithecial formation. The incubation of cultures at 24° for seven days followed by 20° for 14 days, and on media of pH 4 to 6, favored the development of mature perithecia. Although perithecia formed under all light conditions, sunlight apparently inhibited to some extent the delimitation of ascospores.

Random-spore analysis for conidial color (white and olivaceous) and mating type (*A* and *a*) indicated that the characters were not linked. Probably each was conditioned by an allelic gene pair.

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GENETIC AND ENVIRONMENTAL ASPECTS OF FRUITING IN SCHIZOPHYLLUM COMMUNE FR.[1]

JOHN R. RAPER AND GLADYS S. KRONGELB

(WITH 13 FIGURES)

Genetic background is the most critical factor determining both the occurrence of fruiting and the morphology of the fruit bodies in *Schizophyllum commune*. Genetic aspects of fruiting in the Hymenomycetes have received very little attention beyond the qualitative role of incompatibility factors in the heterothallic pattern of mating required for the establishment of the dikaryon (Bensaude, **1917**, **1918**; Kniep, **1918**, **1920**, **1922**, **1923**; Brunswik, **1924**; Quintanilha, **1933**; Papazian, **1951**; et al.). Other genetic work has dealt with anomalies of the fruiting structure (Zattler, **1924**; Greis, **1942**).

It has long been evident, however, that fruiting has requirements in addition to the mating of sexually compatible strains and the establishment of the dikaryotic mycelium. Efforts to encourage fruiting have consisted primarily in providing the dikaryotic mycelium with an approximation of its natural substrate and environment. Many of the Hymenomycetes are wood-rotters, and wood in the form of sawdust or shavings has often been used as the basis for fruiting media (Spaulding, **1905**; Badcock, **1941**) to which frequently have been added various natural products, such as malt or yeast extract, rich in accessory substances (Dickson, **1934**). Various environmental factors, such as light, humidity, aeration, and accumulation of metabolic products, have been intensively investigated and have been considered as having critical importance in the fruiting of a wide variety of forms (Long and Horsch, **1918**; Lambert, **1933**; Dickson, **1934**; Badcock, **1943**; Mader, **1945**; Stoller, **1953**; Plunkett, **1953**, **1956**). Recent papers by Lohwag (**1952**) and Plunkett (**1956**) summarize much of the previous work and review the roles of these several factors in the promotion of fruiting.

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The present paper embodies the results of an investigation on genetic control of fruiting in *Schizophyllum commune*. The experimental results pertain to three aspects of this overall problem: the genetics of the competence to produce fruiting bodies, the inheritance of several abnormalities of fruiting bodies, and the extent to which the inherited pattern of fruiting may be altered by environmental conditions.

MATERIAL AND METHODS

Schizophyllum commune typifies the tetrapolar Hymenomycetes in respect to life cycle and sexuality. Adequate accounts relating to these matters are available (cf. Raper, **1953**), but a brief summary is germane to the present account, particularly as regards the terminology adopted earlier in this work (Raper and San Antonio, **1954**).

With only rare exceptions, fruit bodies (sporocarps) are produced by a dikaryotic (secondary) mycelium formed by the interaction of two sexually compatible homokaryotic (primary, monosporous) mycelia. Mating behavior is determined by incompatibility factors of two distinct series, *A* and *B*, and the dikaryotic-diploid phase is heterozygous for both factors. Incompatibility factors of the two series assort independently at meiosis so that any fruit body yields haploid progeny (basidiospores) of four kinds, each a distinct mating type:

$$A^xA^yB^zB^w \xrightarrow{\text{Meiosis}} A^xB^z,\ A^xB^w,\ A^yB^z,\ A^yB^w.$$

There are many alternate factors at each locus, ca. 340 *A*'s and 64 *B*'s (Raper, Krongelb, and Baxter, **1958**); the number of mating types in nature is accordingly large, and the number of different possible dikaryotic combinations is enormous. This makes it possible to mate strains from nature in practically all combinations.

A propagated monosporous mycelium has been defined as a *strain*, and a specific dikaryotic mycelium formed by the interaction of two strains, a *stock*. *Original* and *laboratory stocks* are defined as dikaryotic mycelia vegetatively propagated from fruit bodies collected from nature and as dikaryotic mycelia resulting from the mating of two strains, respectively. As a matter of convenience, the terms *dikaryotic mycelium* and *dikaryon* will be used interchangeably henceforth in the present paper.

The materials employed in this work were derived as monosporous progeny from 58 original stocks, of which about half originated in the United States. The remaining half came from widely scattered locations all over the world. Most of the stocks were obtained as fruit

bodies freshly collected from nature; a few, as dikaryotic mycelia, were obtained from culture collections where they had been in continuous culture for periods up to 45 years. The sources and collectors of these original materials are indicated in FIG. 1. As they became available, stocks and strains were assigned specific *A* and *B* incompatibility-factor numbers (superscripts) and culture numbers, both identifying series progressing in a roughly chronological order. In the experimental

FIG. 1. The geographical sources and the collectors of the materials screened in the present study. The bracketed numbers preceding each collector's name indicate the number of homokaryotic strains from the location.

work described below, each culture, whether stock or strain, is identified by a culture number. Strains from all 58 stocks were examined in the initial survey, but only a small fraction of these have been used in the subsequent experimental work.

The collections of *Schizophyllum* probably all belong to the single species *S. commune* according to Wm. Bridge Cooke (personal communication), who has examined most of the fruits from which the monosporous strains were derived. Also included in the sample was one stock obtained from the *Centraalbureau voor Schimmelcultures* and originally identified by Linder as *S. radiatum*. Within the sample there was complete interfertility, and it was further shown that the single stock

attributed to *S. radiatum* shared both *A* and *B* factors with numerous other members of the sample.

Two media have been routinely used throughout the work: a *complete medium* consisting of 20.0 g dextrose, 2.0 g peptone, 0.5 g $MgSO_4$, 0.46 g KH_2PO_4, 1.0 g K_2HPO_4, 120 μg of vitamin B_1 (thiamine HCl) and 20.0 g agar per liter of water, and a *minimal medium* differing from the complete only in that dl-asparagine is substituted for peptone. The minimal medium is essentially that originated by Schopfer and Blumer (**1940**). A number of special media used to test the effects of nutritional factors on fruiting will be described in the appropriate sections below. Complete medium has been used except where specifically stated.

The methods and procedures routinely employed were as follows:

1. Fruit bodies from all sources were received in the laboratory in dried condition. These were suspended over an agar medium overnight, or until such time as they were fully revived and discharging spores. When collections were received as dikaryotic mycelia, they were fruited in Petri dishes in the laboratory.

2. A sporulating fruit body, suspended over an agar surface, normally provided a sufficient number of spores within a few minutes. The spores were uniformly streaked over the agar surface with a bent glass rod, and were then incubated at room temperature for 16–24 hours to permit the initiation of germination.

3. Single spores were isolated upon tiny blocks of agar with a needle ground to a cutting edge and sterilized in boiling water. The isolated germlings were routinely transferred directly to agar slants in $\frac{1}{2}$ ounce flat bottles or to agar medium in Petri dishes.

4. Matings were made in standard 90 mm Petri dishes by placing mycelial inocula directly upon the surface of agar medium at a distance of no more than 3 mm apart. When fruiting of the mated pair was desired, only a single mating was made upon about 30 ml of medium per plate; test matings for the determination of mating type were made two per plate upon a thinner layer of medium (ca. 20 ml). In general, minimal medium was used for the former, complete medium for the latter.

5. Cultures were incubated in a laboratory maintained at 22–23° C. Humidity in the laboratory was not controlled, but severe desiccation of plates was not usually evident before 6–8 weeks. It was determined early in the work that although light is required for normal fruiting, neither the duration nor the intensity of light is of critical importance; all cultures have accordingly been maintained under the lighting conditions of the laboratory—30–50 foot candles from fluorescent lamps for

0–9 hours per day. Plates of cultures were usually stacked in piles of ten on the shelves, and only weak diffuse light could reach the bottom plates of sheltered stacks.

6. Experimental lots of dikaryotic cultures were maintained for periods of one to two months and were examined for fruiting at appropriate intervals.

7. To achieve a basis for a meaningful comparison of fruiting between different experimental lots of dikaryotic mycelia it was necessary to establish two criteria relating to the fruiting process: (a) an acceptable definition of fruiting as applied to an individual culture, and (b) a quantitative measure of fruiting in large and often varied samples. The following criteria have been found to satisfy the needs of the present study:

(a) *Fruiting.* Fruiting in a culture is considered to occur at the time of first macroscopic appearance of a hymenial surface in one or more developing fruits. The initiation of sporulation usually follows very shortly thereafter, but the precise time is difficult to determine and impractical to use with large samples.

(b) *Fruiting competence.* The *coefficient of fruiting competence, FC,* has been developed as a quantitative measure of fruiting in large samples of mycelia. This value is based upon both the fraction of cultures fruiting and the time at which fruiting occurs during a 42-day test period as compared to an "ideal" sample in which all cultures had fruited by the fifth day. The ideal sample was a replicated set of 50 cultures of the best-fruiting dikaryon examined in the study. This relationship can be summarized as follows:

$$FC = \frac{100}{K} \int_0^{42} f(F)dt$$

expressed as percent, where $f(F)$ is the rate of fruiting, t, time, and K, a constant defining the fruiting characteristics of the "ideal" sample. Values for FC range from 0, in which no fruiting occurred by the termination of the test, to 100% for the "ideal" sample; higher values are possible but unlikely. The FC value can be calculated directly from the experimental data, but in practice it was more easily determined by measuring with a planimeter the area under a standardized plot of fruiting against time (cf. FIG. 2). Other methods of quantifying fruiting have been explored, such as fruit body weight, rate of spore production, number of fruit bodies, etc., but have been found less satisfactory than the simple temporal relationship implicit in the FC value. The difficulties inherent in these more direct methods of quantification may be illus-

trated by a few facts anticipated from the experiments below: number of fruit bodies per plate, 0-thousands; average size of fruits, 0.5–40 mm; duration of spore production, days to weeks.

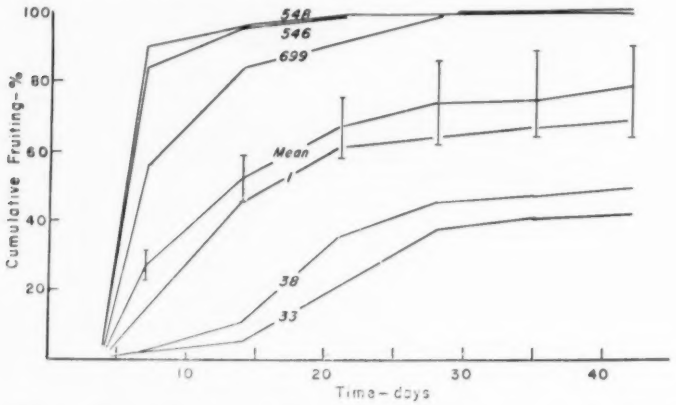


FIG. 2. The pattern of cumulative fruiting of the dikaryons resulting from the cross-mating of 80 homokaryotic strains as compared to dikaryons resulting from the cross-mating of six specific strains each with the remaining 79 strains of the sample. The standard error at successive times is given for the total sample by the vertical bars, the extent of which indicates the extreme heterogeneity of the sample.

Two additional values, both of which are actually incorporated in the *FC* value, have been found to be useful: the fraction of the sample fruiting within the experimental period, or *FF*, and the median fruiting time, i.e., time required for fruiting of 50% of the sample, *MFT*.

8. It has also been necessary to develop a standardized procedure for determining the fruiting characteristics of samples of progeny of specific crosses as a basis for meaningful comparison between different crosses. Progeny from a cross are mated to an appropriate tester that is compatible with all four mating types of the sample. This procedure may be illustrated as follows:

(Parent A	×	Parent B)	Segregants	×	Tester.
A^xB^x		A^yB^y	A^xB^x, A^xB^y, A^yB^x, A^yB^y.		A^zB^z

Since, in this test, the identity of the specific incompatibility factors is of no critical significance, designation of the incompatibility factors

will henceforth be omitted, and the procedure will be written in the following abbreviated form:

$$(699 \times 1)\mathrm{S} \times 38$$

in which the specific parental and tester strains are identified. Numerous tester strains have been used: strains of low fruiting potential to test for fruiting competence, strains carrying specific characters known to be masked by the wild phenotype to test for the same specific characters, and wild-type strains to test for characters that mask the wild phenotype. In general, this method may suffer to a certain degree through the introduction of unsuspected factors in the unrelated tester strain. The method is ideal for the sort of studies reported here, however, since it provides far more information with far less effort than would be possible *via* sib matings.

EXPERIMENTAL RESULTS

All possible matings, several thousand in number, between 116 individual strains of widely diverse origins served as a point of departure and provided both a broad, detailed representation of fruiting characteristics, from which certain central values could be derived, and a number of fruit-body abnormalities that have been utilized in subsequent experimentation.

In this initial series of matings two facts about fruiting competence were evident: (a) there was tremendous variability in the fruiting competence of the large sample of dikaryons examined, and (b) the ability of the dikaryons to fruit and the time of their fruiting were obviously related to the identities of the specific strain components: in certain cases the dikaryons containing a common strain consistently fruited early and well, whereas in other cases the dikaryons containing another common strain might range from early fruiting in some to late or no fruiting in others. These facts strongly suggested that fruiting is genetically determined with the fruiting competence of the dikaryon determined by the component strain having the higher fruiting potential. A quantification of the variability exhibited in the total sample was achieved by a more detailed analysis of 3108 dikaryons resulting from all compatible interactions of 80 strains chosen from the total sample. Fruiting had occurred in 79% of these dikaryons by the end of six weeks. The time of fruiting ranged upward from 5 days. The maximal period during which any of the cultures were observed was 84 days, at which time fruiting still occurred sporadically in a few cultures. The entire sample had an *MFT* value of 13.5 days and an *FC* value of 57.8%. These

fruiting characteristics of the entire sample are implicit in the average plot of cumulative fruiting against time in FIG. 2. Also included in this plot are the standard error limits, the extent of which indicates the extreme heterogeneity of the sample.

Comparative information for a number of individual strains reveals certain of these as differing significantly from the sample average, either as distinctly better or distinctly worse than average. The average for the sample and the characteristics of a number of individual strains are summarized in TABLE 1 and plotted in FIG. 2. For convenience through-

TABLE 1

FRUITING COMPETENCE OF 80 ORIGINAL HOMOKARYOTIC STRAINS

	FF, %	MFT, days	FC, %
548 gf × Sample	98	6	90.5
546 gf × Sample	98	6	88.0
699 gf × Sample	100	7	80.9
Total Sample (upper limit)	91	11	67.9
Total Sample (mean)	*79*	*13.5*	*57.8*
Total Sample (lower limit)	65	16.5	50.0
1 if × Sample	69	16	50.0
38 pf × Sample	49	42+	29.7
33 pf × Sample	42	42+	26.0

out the work to follow, the fruiting of strains or progeny of strains will be classified as *good, gf; intermediate, if;* or *poor, pf,* when their *FC* values lie above, within, or below the limits of standard error of the mean established for the sample, *i.e. good,* $FC > 68\%$; *intermediate,* $FC > 50\%$ and $< 68\%$; and *poor,* $FC < 50\%$.

A. *The inheritance of fruiting competence.* Since the fruiting competence of different strains varies to such a marked extent, the obvious first step to test for inheritance was to mate strains of various levels of competence and to test the progenies of these crosses. The hypothesis of genetic control has as its corollary the expectation that the fruiting competence of the progeny of a given cross should reflect in a regular manner the competences of both the parental strains. The first test comprised the comparison of progenies of crosses between good, intermediate, and poor fruiting strains. The best fruiting sample of progeny was obtained from a *gf* × *gf* cross, and maximal fruiting in this sample obtained when mated with a *gf* tester. Progressive substitution in the series of *if* or *pf* strains, either as members of the cross or as testers, resulted in corre-

spondingly reduced fruiting. These effects are shown in TABLE II, a. The logical and actual terminus of this series is the cross *pf* × *pf*, in which there is failure of the dikaryotic mycelium to fruit.

TABLE II

THE INHERITANCE OF FRUITING COMPETENCE

	Cross	Tester	Sample size	Init. of fruiting, days	FF, %	MFT, days	FC, %
a.	(*699 gf* × *548 gf*)S ×	*546 gf*	121	5	90	6	83.4
	(*699 gf* × *548 gf*)S ×	*38 pf*	121	6	57	31	34.3
	(*699 gf* × *1 if*)S ×	*38 pf*	117	16	40	42+	17.8
	(*548 gf* × *38 pf*)S ×	*33 pf*	114	22	24	42+	9.8
	(*1 if* × *38 pf*)S ×	*33 pf*	112	24	23	42+	7.2
b.	(*699 gf* × *38 pf*)	—	120	6	100	11	78.5
	(*699 gf* × *1 if*)S ×	*38 pf*	117	16	40	42+	17.8
	(*1 if* × *38 pf*)	—	120	16	3	42++	1.3
	(*699 gf* × *38 pf*)	—	120	6	100	11	78.5
	(*699 gf* × *548 gf*)S ×	*38 pf*	121	6	57	31	34.3
	(*548 gf* × *38 pf*)	—	120	6	88	13	64.2

It is evident from these results that there is a definitive correlation between the fruiting competence of the parental strains and that of their offspring. Three additional facts, however, are equally evident: (a) the contribution of the tester strain to fruiting is enormous, as shown by *FC* values of 83.4% and 34.3% for the progeny of a *gf* × *gf* cross when mated with *gf* and *pf* testers, respectively; (b) the progeny of a *gf* × *gf* cross are not uniformly good fruiters, since only slightly more than half of the individuals fruited in matings with the *pf* tester; (c) as *if* and then *pf* strains are substituted for the two *pf* strains in the cross, there is a marked decline in the fruiting potential of the best members of the progenies as shown by the correspondingly longer periods required for any fruiting to occur in the samples.

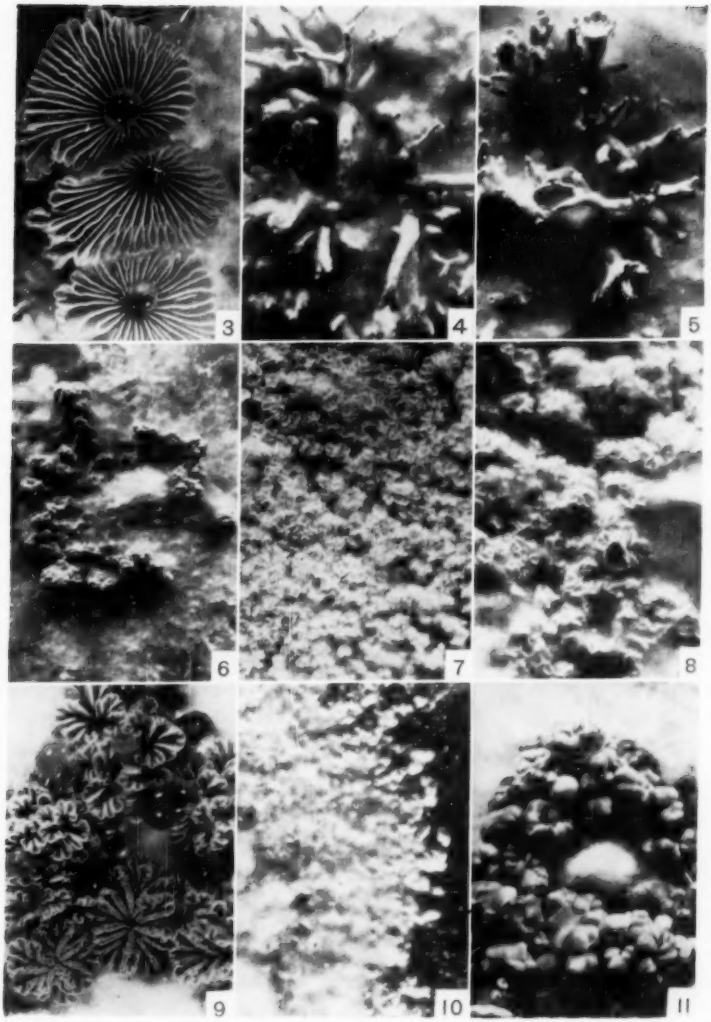
These results demonstrate the genetic control of fruiting competence, but they clearly indicate a complex genetic basis for the inheritance of the character. They leave in doubt, however, certain other relationships of interest: (a) the general low fruiting potential of the progeny of two good fruiting strains, and (b) the exact nature of the contribution made by the tester strain to the measured fruiting competence of a sample of progeny. Information pertaining to these problems was sought in a further series of experiments in which comparisons were made between the fruiting competence of the progeny of each of a number of crosses and that of each parental strain when mated in a replicated series equal

to the number of progeny. A common *pf* tester strain was used for the comparison. The expectation that the fruiting competence of the sample of progeny should lie approximately between those of the two parents was not borne out by the results of the tests. In one case, in a *gf* × *if* cross, fruiting that was intermediate between that of the parents did occur in the progeny, but decidedly below the mean value for the parents. In the other case, *gf* × *gf*, the fruiting competence of the progeny was markedly lower than that of either of the two parents. The results for these two cases are tabulated in TABLE II, b.

Any exact interpretation of these results in terms of genetic models is impossible at the present time. The pattern of inheritance of fruiting competence is of the type generally associated with characters controlled by a number of scattered loci, termed quantitative inheritance, and resembles the inheritance of ear length in maize (Emerson and East, **1913**), kernel color in wheat (Nilsson-Ehle, **1908**), and spotting in the mouse (Dunn and Charles, **1937**). The high incidence of fruiting of dikaryons containing a common *good* fruiting component strain, regardless of the fruiting potential of its mates, indicates the greater potential for expression of good fruiting competence as compared to poor. The high frequency of poor fruiting strains among the progeny of two good fruiting strains, however, suggests the presence even in *gf* strains of numerous unexpressed components which, following reassortment at meiosis, yield new combinations of low fruiting potential. Inter-locus masking is thus exhibited between the components of the complex within a single genome.

B. *The inheritance of fruit abnormalities.* In addition to the heterogeneity of the original large sample of dikaryons in respect to fruiting *per se*, there was an equally striking variability in the morphology of the fruit bodies. No attempts have been made to establish definite limits of normality, but in a small fraction of the dikaryons the fruit bodies were decidedly abnormal by any reasonable criteria. Five particularly striking abnormalities were chosen for study. These were designated *cauliflower*, *medusoid*, *bug's ear*, *coralloid*, and *haploid* because of their gross characteristics, and they are illustrated, along with normal fruit bodies, in FIGS. 3–11.

1. *Cauliflower.* Abnormal fruit bodies quite similar in appearance to those described for *S. commune* by Zattler (**1924**) as *gnarled* occurred in a single mating of the original screening of strains. The two specific strains involved, *546* and *548*, were both progeny of the Overholts I dikaryon, originally collected in Pennsylvania in 1952. These fruits consist of large masses of densely packed mycelium having extremely



FIGS. 3–11. Normal and aberrant fruiting bodies of *Schizophyllum* as viewed from beneath. All approximately × 1 as reproduced here. 3. *Normal.* 4. Extreme *Medusoid,* with no exposed hymenium. 5. *Medusoid,* with numerous, small, exposed hymenial surfaces. 6. *Cauliflower.* 7. *Bug's ear,* each small mycelial fold a distinct fruiting body. 8. Mixed *bug's ear* and *normal.* 9. *Coralloid.* 10. *Microcoralloid,* each fruiting body a microscopic cup lined with hymenium. 11. *Haploid,* one of several distinct forms of haploid (homokaryotic) fruits.

limited and irregular hymenial areas on the exposed (lower) portions; no gills are present (FIG. 6). The fruit bodies called *cauliflower* (*cau*) are somewhat darker than normal; the color is probably the result of the deposition of indigo crystals, since one of the strains involved in the cross is known to produce indigo in culture. Sporulation of fruits is sparse, but most of the spores are viable.

Both the rarity of this abnormality and the normality of the fruits in crosses of these strains to other mates indicated *cau* to be a recessive character. Its inheritance was accordingly tested by crossing one of the parental strains of the *cau* stock with a wild strain and then test-mating the progeny of this cross with the other parental strain, or (*546 cau* × *1*+)S × *546 cau*. A sample of 50 of the progeny of this cross was roughly classified as belonging to three groups: 16 *cau*, 15 *small* (subnormal in size but normal in form), and 19 *wild*. Since these results suggested no simple mode of inheritance, a larger, but similar test was made: (*548 cau* × *699*+)S × *546 cau*. A sample of 120 progeny was tested; fruit body types ranged in a continuous spectrum from *sub-cau*, an abnormality more severe than that of the original *cau*, through various intermediate grades, to *wild*. While the fruit bodies at the extremes were readily distinguishable, the limits of the intermediate classes were indistinct and were arbitrarily chosen. The category *intermediate* (*int*) has been used to describe those forms combining certain features of both *cau* and *wild*—*i.e.* malformed fruit body with gills.

Although the cauliflower of the present study seemed initially to be similar to or even identical with Zattler's *gnarled*, the patterns of inheritance appear to be quite distinct in the two cases. From the F_1 fruits of dikaryotic mycelia resulting from sibling matings between progeny of *wild* × *wild*, *wild* × *gnarled* and *gnarled* × *gnarled*, Zattler concluded that the *gnarled* phenotype was determined by a single-gene, recessive character.

In order to attain a more direct comparison with the results obtained by Zattler, a study was made of the fruit bodies produced by dikaryotic mycelia resulting from the matings of sibling progeny of a *cau* × *wild* cross. The results of these matings may be summarized as follows:

cau × *cau* = only cauliflower fruits
int × *int* = varied, cauliflower to normal fruits
cau × *wild* = only normal fruits
int × *wild* = only normal fruits

One further test gave results that are pertinent to the general problem of the inheritance of *cau:* an analysis of the progeny of a cross be-

tween two strains carrying *cau*. All members of a sample of 120 progeny that were tested proved to be *cau*, although there was some variation in the severity of the abnormality.

From these facts relating to the inheritance and expression of the abnormality, two conclusions can be drawn: (a) *cau* is not a single-locus character but is, like fruiting competence, a quantitative character, as evidenced by the continuous variation among progeny of a *cau* × *wild* cross from severe malformation to normal; (b) *cau* is, unlike fruiting competence, subject to complete phenotypic suppression in dikaryotic combination with mates that do not carry the abnormality.

A number of attempts were made to correlate the inheritance of *cau* with that of other known genetic characters. The following correlations were tested:

The progeny of the cross (*548 cau* × *669+*) were separately tested with *546 cau*, one of the original *cau* strains, and with *38 pf*, a poor fruiting strain. The progeny were grouped into four classes in respect to morphology: *sub-cau*, *cau*, *int* and *wild*, and into three classes according to fruiting competence: *gf*, *if*, and *pf*. A χ^2 test to determine the fit with random distribution in respect to both characters gave the result $P \ll .005$. Corrections for proportionate distribution of the two characters resulted in a lower value of χ^2, which, however, still indicated a significant deviation from expectation, $P < .005$. There thus appears to be a correlation between *cauliflower* and *fruiting competence*. This correlation associates good fruiting with cauliflower morphology [2] and poor fruiting with normal morphology to a greater extent than can be accounted for by chance, but the lack of adequate quantitative expressions for the two characters prevents the calculation of a correlation coefficient. The pertinent data are given in TABLE III.

Among the progeny of several crosses between *cau* and *wild* strains, there was a conspicuous dearth of *wild* strains in certain mating types. This indicated a possible correlation between *cau* and one or both of the incompatibility factors, *A* and *B*, that determine mating type. An analysis of the distribution of *cau* with respect to mating type, comparable to that in respect to the *cau*-fruiting competence above, revealed a barely significant departure from randomness.

Crosses were also made between *cau* and a number of single-locus mutations, each expressed as a morphological aberration of the homokaryotic mycelium; of these crosses only one, that with *felty*, gave evi-

[2] It is interesting to note, in this connection, that *546* and *548*, the two original *cau* strains, were also found to be the best fruiting strains in the original sample screened for fruiting competence.

dence of correlation. In the cross *felty-cau* × + +, 117 progeny were classified as 62 *felty*, 55 *wild*. When these were tested with *548 cau*, 87% of the *felty* siblings which fruited were found also to be *cau*, while 100% of the *wild* siblings which fruited produced normal fruits.

TABLE III

FRUITING COMPETENCE AND FRUIT MORPHOLOGY OF THE PROGENY OF THE CROSS CAULIFLOWER × WILD

		Fruit morphology (tester: *548 cau*)					
		Subcau	*Cau*	*Int*	*Wild*	Total	Freq.
Fruiting Competence (Tester: *38 pf*)	*Good*	5 (5)*	4 (4)	10 (6)	5 (9)	24	.22
	Int	7 (6)	12 (5)	6 (8)	5 (11)	30	.27
	Poor	11 (12)	2 (9)	12 (14)	31 (21)	56	.51
	Total	23	18	28	41	110	
	Freq.	.21	.16	.26	.37		

* Corrected expectation, *i.e.*, (.22 × .21)110 = 5.06.
χ^2 for random distribution = 69.7, $P < 0.005$.
χ^2 for corrected distribution = 29.5, $P < 0.005$.

There is thus fair evidence for a correlation between the *cau* complex, the fruiting competence complex, and *felty;* these relationships cannot be expressed in terms of strict genetic ratios, however, because of the complexity of the two quantitative characters that are involved.

2. *Medusoid.* Another fruiting abnormality, which has been designated *medusoid* (*med*), occurred in varying degrees of severity in numerous matings of the original screening of native strains. The most severe expression of the abnormality was found in the cross *826,* from Connecticut, × *580,* from Illinois (FIGS. 4 and 5). The character is expressed as a large number of thin, elongated projections (presumptive stipes?) which extend downward from the mycelium. Many of these projections do not develop further (FIG. 4), but some open up at their tips to form perfectly normal, if small, fruit bodies (FIG. 5).

The distribution of *med* in the original screenings indicated it to be masked in combination with normal mates but fairly common in strains collected from nature. The tests for inheritance followed the procedure used for *cau* and in general its inheritance resembles that of *cau:* it is clearly a quantitative character, since in no case has it been found to

be transmitted in a simple manner. It differs, however, in one important respect from *cau: med* is either only partially masked in combination with wild type, or components of the character *med* are present in numerous strains that appear otherwise to be perfectly normal.

The limits of the potential for expression of *med* in combination with *wild* may be illustrated by the types of fruits produced in crosses between *med* and presumed *wild* strains. In some cases the fruits are perfectly normal, in others they may be a mixture of normal and medusoid. That they are in no observed case wholly medusoid indicates that the full expression of the character requires the interaction of two strains that carry *med* components (TABLE IV, a).

The complexity of *med* inheritance is shown by the results of the representative crosses given in TABLE IV, b.

TABLE IV

THE INHERITANCE OF MEDUSOID

a. Dikaryotic reactions:

826 med × *580 med* ⟶ Medusoid fruits
826 med × *1019 med* ⟶ Medusoid fruits
826 med × *699 +* ⟶ Normal fruits
580 med × *699 +* ⟶ Mixed medusoid and normal fruits

b. Progeny reactions:

Cross	Tester	Sample	No. fruited	Medusoid	Mixed	Normal
1. (*580 med* × *699 +*)S	× *826 med*	117	78	12	19	47
2. (*826 med* × *699 +*)S	× *580 med*	123	112	96	14	2
3. (*826 med* × *699 +*)S	× *38 +*	123	93	23	35	35
4. (*826 med* × *699 +*)S	× *700 +*	125	103	0	0	103
5. (*826 med* × *1019 med*)S	× *580 med*	116	96	18	43	35
6. (*826 med* × *1019 med*)S	× *699 +*	116	105	1	17	87

3. *Bug's ear.* A third fruit body abnormality, which we have designated *bug's ear* (*bug*), was first seen as the fruiting bodies of *812*, a dikaryotic culture of *S. radiatum* from Panama that had been deposited in the *Centraalbureau voor Schimmelcultures* about 1933 by D. H. Linder. It later appeared in the original screening of the 116 strains in almost all crosses between the two sibling progeny of this stock and the remaining strains of the sample. Bug's ears are microscopic fruit bodies that consist of simple ear-shaped folds of mycelium, borne on the mycelium at a slight inclination from the vertical, with a smooth hymenium covering the shallow, concave, lower surface. The fruit bodies have no gills, but they sporulate profusely (FIG. 7).

The pattern of occurrence of the abnormality in the original screening indicated *bug* to be a dominant character. Its inheritance was accordingly tested as follows: (*833 bug* × *987+*)S × *1+*. A sample of 60 isolates was tested and of these 52 fruited: 25 produced only normal fruit bodies, and 27 produced bug's ear, either solely or mixed with normal (FIG. 8). From these results it was quite apparent that the abnormality is fully expressed in combination with wild type and it appeared as a distinct possibility that it segregated as a single-locus character. The considerable intergradation observed in the sample might then be the result of modifying factors, or alternatively, it might indicate yet another quantitative character.

In an effort to resolve the uncertainty of 1- *vs.* multi-locus determination, a second-generation test was made using isolate *56* of the previous cross as a parental *bug* strain: |(*833 bug* × *987+*)*56 bug* × *1+*|S × *699+*. Fifty isolates of the progeny of this cross were collected and all fruited with the wild tester. The fruiting bodies of these matings could be classified in respect to morphology and to average size and range as follows: 15 uniformly bug's ear, average fruit size, 1.04 mm, range, 0.6–1.4 mm; 10 varied mixtures of bug's ear and normal, average fruit size, 2.8 mm, range, 2.4–3.5 mm; 25 normal, consisting of 9 small (4.2, 3.1–5.5 mm) and 16 large (5.8, 3.8–10.4 mm). The 15 classified as *bug* were thus well isolated from the remainder of the sample, but otherwise the progeny could not be grouped into well characterized classes. As before, however, the abnormality occurred in half the progeny.

Precisely the same results have been obtained in three further crosses and in a series of five successive generations of backcrosses to a wild type strain. In the fifth backcrossed generation, a sample of 192 was tested, and of these, 101 produced bug's-ear fruits (85 bug's ear and 16 mixed) and 91 produced only normal fruits (TABLE V). These results strongly suggest a basic 1-locus determination of the abnormality. It is equally evident, however, that some modifying factor is involved. Further tests now in progress should clarify the modification of the *bug* character.

4. *Coralloid*. The fruit bodies of culture *617*, a dikaryotic stock collected in New Guinea in 1945 and since maintained in the fungal culture collection of the Quartermaster Corps, are of a distinctly abnormal type that we have termed *coralloid* (*cor*) (FIG. 9). The fruit body is compact, markedly convoluted, and somewhat darkened, probably by the deposition of indigo (see below). Sporulation is not impaired by the abnormality. The fruiting bodies of the dikaryotic stock deposited in

the *Centraalbureau voor Schimmelcultures* by Kniep now appear similar or identical to *coralloid*, but no analysis has been made of this material.

As a preliminary test for the expression of *cor*, one of the progeny of the original dikaryotic stock was mated to 12 different strains that severally carried *cau*, *med*, good fruiting competence, and several mycelial mutations. The fruit bodies were coralloid in every case, and

TABLE V

THE INHERITANCE OF BUG'S EAR

		Bug's ear			
	Sample	Pure	Mixed	Normal	χ^2
a. (*bug* × +)S × +	52	18	9	25	.07
(*bug* × +)S × +	50	15	10	25	0
(*bug* × +)S × +	39	14	4	21	.22
b. Backcrosses					
Generation					
(*833 bug* × *699* +)S I	38	22	0	16	.94
II	[62	33	10	17	12.60]*
III	56	18	6	32	.62
IV	62	24	8	30	.06
V	192	85	16	91	.74
Total	489	196	53	240	.16, P = .75
		249			

* Excluded from total.

fruiting occurred in all of the matings within 7–10 days. These interactions suggested *cor* to be a dominant character, possibly correlated with good fruiting competence.

A sizable sample (251) of progeny of the original dikaryon was then collected and found to comprise two distinct types in equal frequency: *wild* and a mutant mycelial form (*mut*) that produced a blue pigment (*blue*), since identified as indigo. This sample was tested both for coralloid morphology and for fruiting competence by mating with *38* + *pf*, a morphologically normal strain of low fruiting potential. Without exception among those that fruited, the *blue-mut* strains yielded coralloid fruits. Furthermore, the frequency of fruiting was higher and the time of fruiting was earlier for the *blue-mut* segregants (TABLE VI, a). In the dikaryons produced by the interaction between *mut* and wild-type strains, all three characters, *cor* fruits, pigment production, and mycelial aberration, are unmistakedly expressed.

The segregation of the mutant phenotype among the progeny of the original dikaryon yielded a very good 1:1 ratio (P = .60). Two additional facts, however, indicated caution in equating this ratio to a single-locus character: the very low recovery of isolated progeny, 62%, could mean a badly skewed sample, and considerable variation among the mutant progeny could indicate the implication of additional genetic factors. The progeny of two additional series of crosses have been analyzed to clarify the inheritance of the coralloid abnormality. These

TABLE VI

INHERITANCE OF THE CORALLOID-MUTANT-BLUE COMPLEX

Tester	Sample	Myc. morph.	No.	Fruit morph.	FF, %	FC, %
a. (New Guinea Dik., *617*)S × *38* +	251	Mutant	115	Coralloid	99.1	92.2
		Wild	126	Normal	77.3	64.9
				$\chi^2 = 0.32$, P = .60		
b. Five crosses summed: [(*617*)S *cor* × *699* +]S × *38*	356	Mutant	145	Coralloid	95.9	84.0
		Wild	211	Normal	50.2	35.8
				$\chi^2 = 8.1$, P = .001		

c. Segregation of mycelial morphology:

[(*617*)S *cor* × *699* +]S	Cross	Sample	Wild	Mutant	χ^2	P
	1.	735	361	374	.23	.60
	2.	432	223	209	.45	.50
	3.	523	274	249	1.19	.28
	4.	673	328	345	.43	.50
	5.	717	364	353	.17	.65
		3080	1550	1530	.13	.66

series involved (a) crosses between 5 different *cor* offspring of the New Guinea stock × *wild*, and (b) successive backcrossing for four generations between a typical *cor* strain × *wild*. Recovery in all of these crosses was consistently above 90%, variation in the mycelial aberration among the mutant progeny was much less than in the original samples, and the association of the three characters of the coralloid complex was absolute: no single exception occurred among the 1220 progeny tested.

In all of the crosses of series *a* (TABLE VI, b) and the first two generations of series *b*, a total of 916 isolated progeny were scored as 360 *blue-mut*:556 *wild*. The examination of the germinating spores of the third backcrossed generation at 48 hours after sowing (24 hours after isolations were routinely made), however, revealed two morpho-

logically distinct types of germlings. In this and in the succeeding generation, a total of 257 germlings were simultaneously isolated and scored as *mut* or *wild* with only 7 mistakes—an accuracy of 97.3%. Counts were subsequently made on random samples of germling progenies of five additional crosses between *blue-mut* segregants of the original *cor* dikaryon and *wild*. The results in each case showed a 1:1 segregation (TABLE VI, c).

Although these results clearly demonstrated the *blue-mut-cor* complex to segregate as a unit, the series of backcrosses revealed an unexpected complication: there were progressive detrimental changes both in fruiting competence and in the morphology of the fruit bodies. The fruit bodies in the terminal generation, IV, were microscopic, *micro-coralloid*, with spore production reduced to a negligible fraction of the original quantity (Fig. 10). These effects, however, obtained only in matings with the particular wild-type strain used in the backcrosses. The same series of *cor* strains mated with three other wild-type tester strains produced only typical coralloid fruits with one tester, small, intermediate coralloid fruits with another, and only micro-coralloid fruits with the third—regardless of the origin of the *cor* strains in the backcross series. It is thus evident that the expression of the single locus *cor* is strongly modified by some additional factor(s), for the clarification of which further studies are planned.

The production of indigo in all *cor* strains is evident only after a protracted period of vegetative growth, and the character may consequently be classified as a *late-blue* (Raper and Miles, **1958**). Since two *late-blue* loci, *late-blue-1* linked to the *A*-incompatibility factor and *late-blue-2* linked to *plumy* and *felty*, are known to result in delayed indigo production and since no evidence had been found of linkage to the *A* factor, it appeared desirable to test the linkage of *cor* with *plumy*. The two loci are closely linked: among 400 progeny of two *cor* × *plumy* crosses, only a single wild recombinant was recovered. The more adequate resolution of the *blue-mut-cor* complex, whether as a single pleotropic locus or as three very closely linked loci, and the possible identity of the two mycelial characters associated with *cor* as *felty* and *late-blue-2*, will be attempted as time permits.

5. *Haploid (homokaryotic) fruiting.* Although the fruiting of unmated, haploid strains is not strictly a fruit body aberration, some mention should be made here of fruiting in homokaryons. Haploid fruiting bodies have been reported in *Schizophyllum* by Wakefield (**1909**) and *Kniep* (**1920**) but are of rare occurrence in the materials used in the present study. Haploid fruiting strains have been found only among

the progeny of four dikaryotic stocks maintained in culture for long periods, *813* from Germany (Kniep), *526* from Pennsylvania (Overholts), *812* (*S. radiatum*) from Panama (Linder), and *965* from France (Heim), and from one stock, *815*, recently collected from Australia.

The morphology of haploid fruits in strains of different origins varies widely and ranges from only slightly stunted, gilled structures to amorphous masses of tightly compressed hyphae (FIG. 11). Sporulation is very scarce in all cases, the germinability of the few spores produced is very low, and of those that germinate only very few develop beyond the germling stage. It is accordingly difficult to obtain adequate samples of progeny of haploid fruits for extensive analysis.

In each case tested to date, the progeny of the haploid fruits have been of the single mating type corresponding to that of the parental strain. Only one strain has been tested beyond a single generation; in this, 38 monosporous mycelia were recovered from 128 germlings. These were all of the same mating type as the parent and 66% of them produced haploid fruits. Twelve monosporous mycelia were recovered in the next generation; mating type, mycelial morphology and frequency of fruiting were again the same as in the parent. The haploid nature of these fruits thus seems to be securely established.

A number of tests have been made with haploid strains for the purpose of testing (a) the inheritance of haploid fruiting and (b) the relationship between haploid and dikaryotic fruiting competence. A comparison of this kind has been made for *Lenzites trabea* by Barnett and Lilly (**1949**), who found an increased frequency of fruiting in dikaryotic combinations constituted of haploid fruiting strains; these authors concluded that the same factor(s) might be responsible for haploid and dikaryotic fruiting.

Data relating to the two points of particular interest have been obtained for three *hap* strains of *Schizophyllum* as follows:

(a) The dikaryotic fruiting competences of the three haploid strains as determined in replicated sets of matings with 38 *pf* were *360 hap, gf, FC* = 94.3%; *357 hap, if, FC* = 59.4%; and *359 hap, pf, FC* = 36.2% (TABLE VII, a).

(b) The haploid fruiting competences of the progenies of two *hap* × *hap* crosses (as revealed by the spontaneous fruiting of the monosporous mycelia set out individually on fruiting medium) were: (*359 hap* × *360 hap*)S, good, *FC* = 79.1%, and (*357 hap* × *359*)S, poor *FC* = 7.3% (TABLE VII, b).

(c) The dikaryotic fruiting competences of the progenies of the same two *hap* × *hap* crosses as tested in matings with *38 pf* were (*359*

hap × *360 hap*)S, poor, *FC* = 37.8%, and (*357 hap* × *359*)S, poor *FC* = 25.6% (TABLE VII, c).

A number of interesting points are revealed by these data. (a) There is obviously no necessary correlation between good haploid and good dikaryotic fruiting; only one of the three haploid strains chosen for study ranks as a good dikaryotic fruiter, whereas all three routinely fruit spontaneously in isolation. This lack of correlation would indicate

TABLE VII
HAPLOIDY

a. Dikaryotic fruiting competence of haploid *strains:*

	Sample	FF, %	MFT, days	FC, %
(*360 hap* × *38 pf*)	50	100	6	94.3
(*357 hap* × *38 pf*)	50	88	18.5	59.4
(*359 hap* × *38 pf*)	50	36	42+	36.2

b. Haploid fruiting in progeny of *hap* × *hap:*

	Sample	FF, %	MFT, days	FC, %
(*359 hap* × *360 hap*)S	51	48	42+	37.8
(*359 hap* × *357 hap*)S	32	10	42++	7.3

c. Dikaryotic fruiting in progeny of *hap* × *hap:*

Tester	Sample	FF, %	MFT, days	FC, %
(*359 hap* × *360 hap*)S × *38 pf*	51	98	7	79.1
(*359 hap* × *357 hap*)S × *38 pf*	32	38	42+	25.6

different genetic bases for fruiting under the two different circumstances. (b) The fraction of haploid fruiters in the progeny of each of the two crosses is surprisingly low in view of the strong haploid fruiting potential of both parents in each cross. There can be no doubt, however, of the inheritance of the character, for the frequency of its expression in the progeny of these crosses is enormously greater than in the progenies of *wild* × *wild* crosses. Haploid fruiting strains have been isolated only from the sources listed above. None has been recovered among a conservatively estimated sample of 15–20,000 isolates from crosses involving strains of other origins. That the phenotype comes to expression only in a portion of the progeny of such *hap* × *hap* crosses indicates a plurality of determining loci, *i.e.*, yet another quantitative character. (c) Two additional points established here are of doubtful significance in view of the relationship stated above in (a): The very good fruiting competence

of the progeny of (*359 hap* × *360 hap*)—the highest for any sample of progeny examined to date—and the rough parallel between haploid and dikaryotic fruiting in the two crosses.

A final point of undoubted significance follows from an observation relating to haploid *vs.* dikaryotic fruiting of the individual members of progenies of the two *hap* × *hap* crosses. There was no apparent correlation between the fruiting of individual strains as haploids and as dikaryons in respect to occurrence or to time of fruiting. Much further work will be required to define the genetics of haploid fruiting satisfactorily.

C. *The effects of environmental conditions upon fruiting.* The effect of environmental conditions upon fruiting in a wide variety of Hymenomycetes has been the subject of much experimental work. The external factors tested have included light, nutrients, atmospheric conditions, humidity, temperature, and toxic substances. In the present work, limited studies have been made of the effects of certain of these factors to determine their effects upon fruiting *per se* and upon the expression of certain inherited abnormalities.

1. *Light.* Light has been implicated in different ways in the fruiting processes of a number of different species (Buller, **1909**; Long and Horsch, **1918**; Dickson, **1934**; Badcock, **1943**; Plunkett, **1953**). In the present work a simple series of experiments was performed to test whether (1) light is necessary for fruiting, (2) light is required for normal fruit morphology, and (3) light, by proper manipulation of duration and intensity, can enhance fruiting in genetically determined poor fruiting combinations. For these tests, two dikaryotic mycelia that gave normal fruits were used: *1 if* × *38 pf*, a poor fruiting combination, and *546 gf* × *14 if*, a good fruiting combination. A replicate set of 10 freshly mated cultures of each of these dikaryotic mycelia was maintained for 30 days under each of the following conditions of illumination: (1) continuous darkness except for a brief daily examination under red light of low intensity, (2) continuous fluorescent illumination at an intensity of 50–70 foot candles, (3) continuous fluorescent illumination at 1–2 foot candles, and (4) alternating dark and light at 50–75 foot-candles, both for 8–16 and for 16–8 hours of dark and light, respectively. The results of these four different conditions of illumination were as follows: (1) all replicates of the *gf* × *if* cross fruited, but about one day later than usual and with smaller fruit bodies. None of the *if* × *pf* mycelia fruited. (2) and (3) all *gf* × *if* fruited normally by the fifth day; no fruiting of the *if* × *pf* mycelia, (4) all *gf* × *if* replicates fruited by the sixth day; no fruiting in *if* × *pf* replicates.

It therefore appears that light is not required for fruiting but it is required for normal fruiting, although the intensity and duration of light do not appear to be critical. It appears unlikely that the condition of illumination could induce fruiting in a genetically determined poor fruiting combination.

2. Thiamine concentration. The requirement for thiamine, both for vegetative growth and for fruiting, has been generally accepted for *Schizophyllum* and many other wood-rotting Hymenomycetes since the publication of the work of Robbins (**1938**) and Schopfer and Blumer (**1940**), which called attention to the wide distribution of this deficiency among the fungi. No specific study has been reported, however, of the quantitative relationship between thiamine and fruiting, nor of the variability of the requirement in different dikaryotic stocks. In the latter connection it was previously found (Raper and San Antonio, unpublished) that the thiamine requirement for growth varied tremendously among a group of 17 different wild-type strains collected from nature.

In a preliminary experiment, a number of poor fruiting dikaryons were grown on glucose-asparagine medium supplemented with thiamine in concentrations varying from 0–180 μg/liter. Fruiting occurred in none of the cultures by the end of a month, and it was concluded that thiamine is not a limiting factor in fruiting of poor combinations. Two additional series of experiments have since been performed. In the first, a good fruiting combination, *699 gf* × *2 if*, was grown in replicate sets of 10 cultures on glucose-asparagine medium supplemented separately with 0, 1.8, 7.5, 30 and 120 μg/liter of thiamine and with the same quantities of 2-methyl-5-ethoxymethyl-6-amino pyrimidine, the pyrimidine moiety of thiamine. On the unsupplemented medium there was only very sparse growth, while at the 1.8 μg level of both supplements there was good vegetative growth but no fruiting. Fruiting occurred at all higher concentrations on both thiamine and pyrimidine, with the earliest and best fruiting at 7.5 μg thiamine. In general, fruiting was earlier and better on thiamine than on pyrimidine; in the pyrimidine series the best results were obtained at 30 μg.

In the second series, eleven stocks of varying fruiting competence were grown on media supplemented with 1.8, 3.75, 7.5, 15 and 30 μg/liter of thiamine. The various dikaryons reacted differently, with earliest and best fruiting in 6 stocks at 30 μg, in 3 at 15μg, and in 2 at 7.5 μg. No fruiting occurred at 1.8 μg.

These results thus confirm thiamine as a necessary factor for fruiting in *Schizophyllum*. They further show, however, that the requirement is variable for different stocks, that it cannot be wholly met by the

pyrimidine moiety alone, and that thiamine, while necessary, is not sufficient to induce fruiting.

3. *The effect of other growth supplements and growth inhibitors.* Since 1905, when Spaulding cultured *Schizophyllum* on an agar-solidified infusion of green wood, media employing various rich natural substances have been used for the growth and fruiting of this and other hymenomycetous fungi. The extensive literature on the subject is reviewed by Lohwag (**1952**). In 1940 Schopfer reported a satisfactory minimal medium for *Schizophyllum*, and in the present work, Schopfer's minimal has been found to be very good for vegetative growth and, somewhat surprisingly, for fruiting as well. It still remained, however, to find the relative merits of various media in promoting normal fruiting in minimal time. Toward this end a number of diverse media have been tested with a variety of dikaryons.

The causal basis for the several abnormalities described above is a matter of some interest, and the possibility of responsibility residing in specific nutritional deficiencies was examined. Twenty-four dikaryons, comprising 11 that contained *cau* and varied in morphology from cauliflower to normal, 9 that produced medusoid fruits, and 4 that produced pigmented fruits, were grown on (a) minimal medium as control, (b) complete medium plus 5 g/liter each of yeast extract and malt extract, and (c) minimal medium plus 120 mg/liter rose bengal. The medium containing rose bengal, one of the most widely used mycelium inhibitors, was included because restricted mycelial growth appeared to characterize a number of dikaryotic stocks of high fruiting competence. In some cases the cultures exposed to rose bengal fruited earlier and more profusely than the controls; the addition of yeast and malt extracts not only failed to enhance fruiting, but in many cases significantly retarded it, an effect recently reported by Plunkett (**1953**) in *Collybia velutipes*. In no case was there any detectable difference in the morphology of the fruit bodies on the three media.

The apparent enhancement of fruiting by rose bengal prompted a test of the effect of various mycelium-inhibiting substances upon fruiting. A poor fruiting combination, *1 if* × *38 pf*, was grown on minimal medium and on a series of minimal media to which were added the following, each at the concentration of optimal vegetative inhibition: 0.0124% rose bengal, 0.0175% tergitol-7, 0.07% aerosol, 0.117% dehydrocholic acid, 0.017% sodium lauryl sulfate, 0.248% sodium glycocholate, 0.07% desoxycholic acid, and 0.1% sodium bisulfite. The earliest and best fruiting occurred on tergitol-7, aerosol, and sodium lauryl sulfate, although on none of these media was fruiting better than very sporadic. Sodium

lauryl sulfate gave the best mycelial restriction; it was tested in other concentrations with no further enhancement of fruiting. The summed results of the various tests with mycelial inhibitors indicated only negligible effect upon fruiting.

In the examination of a large number of stocks and strains of *Schizophyllum*, it became increasingly evident that, in general, dikaryotic cultures that had been kept in culture for a long time tended to fruit better than those recently collected from nature. In an effort to validate this general impression and to compare a number of dikaryons on different media, five dikaryons recently collected from nature, two ancient dikaryons, and two poor-fruiting laboratory dikaryons were grown in

TABLE VIII

FRUITING OF DIKARYOTIC MYCELIA UPON DIFFERENT MEDIA

Stock	Date	Minimal		Min. + yeast		Min. + Na-lauryl-SO_4	
		No.	(5) Time, days	No.	(5) Time, days	No.	(10) Time, days
817	1956	0		0		10	12.5
815	1955	0		0		3	13.0
816	1956	0		0		9	22.0
638	1955	0		0		7	10.4
636	1955	5	5.4	1	5.0	10	11.4
617	1945	5	7.0	5	8.0	10	19.5
815	1920	5	7.0	5	7.0	10	11.0
(*1 if* × *38 pf*)		0		0		0	
(*33 pf* × *38 pf*)		0		0		0	

replicate sets of 5 cultures on (1) minimal medium, (2) minimal + 5 g yeast extract, and 10 cultures on (3) minimal + 0.017% sodium lauryl sulfate. The fruiting of these 9 dikaryons fell into three distinct patterns: (a) Four of the five new dikaryons fruited only on the medium containing sodium lauryl sulfate. (b) One of the new dikaryons and both of the ancient ones fruited on all three media; fruiting occurred at about the same time as group (a) on sodium lauryl sulfate but much earlier on the other two media. (c) The two poor-fruiting dikaryons fruited on none of the three media (TABLE VIII).

In general, these results support the impression of better fruiting in old than in newly collected dikaryons, in spite of the fact that one of the newly collected ones behaved like the long-cultured stocks. No rationale can be seen at the present time to account for the retardation of fruiting of the good-fruiting stocks on sodium lauryl sulfate.

4. *Mechanical injury, etc.* Previous reports of experiments on fruiting in hymenomycetous fungi have intimated or stressed the beneficial effect of crowding, mechanical barriers, mechanical injury and contamination with other micro-organisms (Wakefield, **1909**; Corner, **1932**; Roshal, **1953**). A number of experiments have been performed with *Schizophyllum* to test certain of these factors; the results have been sporadic and inconclusive.

Multiple inoculation of a dikaryon leads to early crowding, but no effect on the time of fruiting was observed. The technique does, however, provide an efficient means of obtaining large quantities of fruiting bodies simultaneously.

Many dikaryons typically fruit at the edge of the plate following the cessation of vegetative growth. The removal of a peripheral ring of medium with or without the outer fringe of the mycelium, however, has only questionable effect on the time or quantity of fruiting.

Inoculation of the outer portion of the medium upon which a dikaryon is growing with *Bacillus mycoides* has a rather spectacular effect on fruiting in certain cases. This effect obtains, however, only for intermediate fruiting dikaryons on media in which thiamine is limiting, and the effect almost certainly depends upon the superior synthetic ability of the bacteria.

Mechanical damage to the mycelium appears in certain cases to enhance fruiting, but in a number of trials in which replicated sets of dikaryons were lacerated, no significant increase in fruiting was evident in comparison with undisturbed controls.

6. *Stagnant, humid air.* No specific experimentation has been done to determine the effect of humidity and composition of the atmosphere on the development of fruit bodies. The routine practice of growing fruiting dikaryons in unsealed Petri plates apparently provides sufficient gaseous exchange between the cultures and the atmosphere to permit normal development. On one occasion in a replicate set of cultures of a dikaryon of known high fruiting potential which was sealed with masking tape for a period of 4 weeks, the average number of fruit bodies per plate was decreased and the form of the fruits abnormal—most of them strongly resembled cauliflower. That marked abnormality of the fruit bodies should develop in the humid, CO_2-laden atmosphere of sealed plates is entirely consistent with Plunkett's (**1956**) demonstration of the effect of excessive humidity and staled air on the development of fruits of *Polyporus brumalis* and *Collybia velutipes*. Other environmental factors, widely varied, could doubtlessly affect the fruit form in *Schizo-*

phyllum in a similar manner; such additional factors, however, have not been encountered in the present work.

In the present study, when progeny from a particular recalcitrant stock have been needed, the replication of cultures—the multiplication of low probabilities—has been the most rewarding approach. No reliable means has been found to induce fruiting in dikaryotic stocks constituted of strains of low fruiting competence.

DISCUSSION

The experimental results presented here can leave no doubt of the primary importance of genetic determination in the fruiting process of *Schizophyllum*. Yet, the fact of prior genetic requirements for fruiting in no way discounts the importance of the proper environmental conditions. The effects of the latter can only be expressed upon a genetically predetermined, favorable system, and the relationship between the roles played by genetic and environmental factors is thus one of chronology rather than of relative importance. The failure in the present work to enhance fruiting of genetically inadequate dikaryotic stocks and to restore morphological normality to genetically determined aberrant forms is considered only as indicative of this basic sequence of determining factors.

That tremendous variability in all aspects of the fruiting process exists in presumably normal strains of hymenomycetous fungi should surprise no one. That no one has looked for such variability and more particularly that certain authors have disregarded wide variability in their materials while engaged in a study of the effects of environmental factors on fruiting is somewhat surprising. In many cases, moreover, only single dikaryotic stocks have been the subjects of investigation, and their fruiting requirements and characteristics have been generally attributed, explicitly or implicitly, to the entire species to which they belong. The size of the sample examined here provides a far more comprehensive representation of a single form than has been achieved in any previous work in the Hymenomycetes. The greater appreciation of the fruiting process thus achieved, however, serves to emphasize numerous newly revealed problems, certain of which deserve brief consideration.

The complex nature of most of the genetic characters shown to be involved in fruiting stands in striking contrast to the prevalence of simply segregating, one-locus characters that determine morphology and biochemical activities in the homokaryotic mycelium. Of some 35 tested characters affecting growth, form, and mating behavior of the homo-

karyotic mycelium, all but four segregated as single loci; of these exceptional four, two, the *A* and *B* series of incompatibility factors, relate to the initial phase of the progression that leads to fruiting (Raper and Miles, **1958**). Two different lines of rationalization might account for this apparent distinction.

The first and perhaps the more probable reason follows from the specific methods of screening that have been used in the present study. In the screening of the 116 homokaryotic strains *via* all possible cross matings, a number of fruiting abnormalities were found. Of these, only two, *cau* and *med*, have been shown to be phenotypically suppressed in combination with wild type and both of these are complex quantitative characters. *Med* is apparently a relatively common abnormality and was found in a number of crosses between strains of diverse origins; *cau* was found only once and then by accident: the two monosporous progeny of Overholts I chosen for the tests happened to carry the character. The procedures used would reveal, at best, only a quarter or so of such rare masked characters actually present in the original stocks. Numerous sib matings among the F_1 progeny of each of the stocks would be necessary to find all of the masked or recessive characters present in the materials, and a search of this kind is planned as an extension of the present study. The use of the total-sample testing method developed and employed here, however, appears as superior to the sib-mating method for the detailed analysis of any type of genetic character affecting the fruiting process. It requires far fewer matings for the complete analysis of the sample of progeny, and it completely avoids all of the complexities introduced by the tetrapolar pattern of mating—including the possibility of complete failure of the analysis due to close linkage of the character under study with one of the incompatibility factors.

Only a single previous investigation with *Schizophyllum* has related directly to those aspects of its biology considered here. Zattler, in 1924, published the results of an extended study on the inheritance of an abnormality of fruit bodies, *gnarled* (*Knäuel-Fruchtköpfer*), which so closely resembles the abnormality here termed *cau* as to raise the question of their possible identity. The results obtained by Zattler appear to leave no doubt of the 1:1 segregative pattern of *gnarled*.[3] That *cau*,

[3] The same information could have been achieved, however, with a small fraction of the tests that were actually made. Zattler used sib matings throughout his study; for the initial analysis of a sample of 84 sibs he made 424 crosses (of 954 possible compatible combinations) *plus* the necessary 336 matings for the determination of the mating types of all members of the sample. Total-sample testing would have required only about 100 matings (84 test matings *plus* 15 or 20 for the necessary backcross and the identification among its progeny of a suitable tester strain).

as analyzed here, is not a single-locus character is equally clear. Two alternative possibilities might apply: (a) *gnarled* and *cau*, although phenotypically similar or even indistinguishable, are genetically distinct or (b) *cau* represents *gnarled* as affected by numerous independently assorting factors. The latter possibility will be examined *via* several successive generations of backcrosses against a normal strain to determine if *cau* can be reduced to a simply segregating character.

The alternative rationalization of the distinction of simple *vs.* complex genetic characters in homokaryotic and dikaryotic mycelia, respectively, follows from the different ways in which natural selection might operate in the two types of mycelia. In the former there is minimal opportunity for the accumulation of the numerous, individually unimportant components of the typical quantitative character; the problems of survival are too immediate for long-range and complex adaptive change. In the balanced dikaryotic association, by contrast, each genetic change that adds towards a dominant beneficial character has an immediate selective advantage, while at the same time genetic complementation masks, hence permits the perpetuation of, component elements of unexpressed deleterious characters such as the *cauliflower* complex.

Actually, on the basis of the examination of the materials available, the suspicion cannot be avoided that selection for high fruiting competence and selection for complex deleterious characters are in some manner connected. Among a number of dikaryons that have been maintained for protracted periods in culture, all but one produce fruits early and in abundance, yet almost all of them are burdened with mutant characters that seriously affect both mycelial growth and the form of the fruits that are produced. To the extent that these aberrations have been tested, the former are predominantly single-locus characters, *felty, concentric, pigmy,* etc. (Raper and Miles, in press), whereas the latter are typically either masked quantitative characters, *cau, med* and *hap,* or dominant, single-locus characters that appear to be associated with modifying factors, *bug* and *cor.* A better understanding of these matters is being sought in a continuing study (Miles, unpublished).

That the burden of mutations in ancient dikaryotic mycelia has been acquired in culture appears certain for several reasons: mutant progeny have rarely been found in the present study to originate from recently collected stocks, and where such aberrations have been found they have occurred singly; the multiplicity of mutant characters now known to be carried by the Kniep stock, deposited in the *Centraalbureau voor Schimmelcultures* about 1922, would not likely have been overlooked by that most careful and perceptive investigator; two lines of the Overholts I

stock, separately cultured for only five years, differ in the mutations that they contain (Miles, unpublished). Yet the vegetative mycelia of these stocks appear relatively normal and differ from recently collected stocks only in extent of growth and in the fruiting process: the early production of fruits and, in some, the aberrant nature of the fruits—*i.e.*, *bug* in the Linder stock of *S. radiatum* and *cor* in the Kniep and New Guinea stocks.

It appears entirely possible that all of these characters of the ancient dikaryotic stocks may have resulted from the fact that they have been vegetatively propagated, with the consequent prevention of the natural selective processes normally associated with fruiting, through which deleterious genetic alterations are eliminated. Early fruiting is such a convenience in the laboratory that it is all too easy to attribute to it a high natural selective advantage, although there is almost invariably an accompanying, and sometimes severe, restriction of growth of the parental dikaryotic mycelia. For fungi growing upon natural substrates, without the artificial limitations of culture dishes, etc., prolonged vegetative growth of the dikaryotic mycelium and the later production of more and larger fruiting bodies may far better serve the continuing biological needs of the species than does early fruiting. To the extent to which the evidence of the present work is applicable, natural selection appears to operate toward less avid fruiting than displayed by the ancient dikaryons: with negligible exceptions, dikaryons isolated from fruits collected from nature are indifferent fruiters according to the criteria and under the conditions adopted for the present study.

The phylogenetic implications of certain of the fruiting abnormalities are of some interest. Three of these fruits would certainly not be recognized as belonging to *Schizophyllum* in the absence of knowledge of their origins, *i.e.*, *cau*, *bug*, and *micro-cor*. The latter two are dominant characters and, depending upon their frequency in the natural population, must come to expression in the natural environment. We have found them only in dikaryotic stocks maintained for long periods in culture, but they are easily transferrable, *via* cross matings, to all stocks of *S. commune*. The development of bug's ear and micro-coralloid is completely arrested at the simple, shallow-cup stage which marks the initial hymenium-bearing stage in the early development of normal fruits prior to the appearance of the radial ridges that eventually develop into gills. In either case, these simple, hymenium-bearing cups would appear to have rather definite significance in the decades-old controversy on the natural affinities of the Schizophyllaceae—whether as allied with the

Cyphellaceae of the Polyporales or as a constituent family of the Agaricales (Gäumann-Dodge, **1928**; Heim, **1948**; Singer, **1951**, *et al.*).

The fruiting process in the Hymenomycetes has long been one of the more intensively studied problems among the fungi. The study reported here has placed primary emphasis upon a previously unexplored aspect of this problem. The results that have been achieved have the very practical importance of permitting routine and dependable genetic analysis of factors affecting all stages in the developmental cycle. The demonstration of genetic control of fruiting has furthermore provided the necessary information to permit, *via* selective breeding, the fruiting of laboratory stocks carrying any desired combination of characters. These practices will undoubtedly prove equally useful with many other species of the higher fungi. Beyond this practical matter, however, the fact of critical determination of numerous aspects of the fruiting process by genetic factors provides valuable new means of study and analysis. At the same time, the fruiting process is revealed as a far more complex phenomenon than has been previously recognized. The findings reported here would thus seem to indicate a new dimension for future inquiry on fruiting in the Hymenomycetes.

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SUMMARY

An extensive study of fruiting in a large, world-wide sample of *Schizophyllum* has revealed genetic background as the most critical factor in determining the occurrence and time of fruiting and the form of the fruiting bodies.

In a sample of 116 homokaryotic mycelia cross-mated in all combinations, fruiting occurred in 80% of the resulting 6546 dikaryons. The occurrence and time of fruiting, however, were not random: good fruiting and poor fruiting were conspicuously correlated with specific component strains. Detailed analysis of a smaller sample of 80 strains permitted a quantitative determination of *fruiting competence* and the classification of individual strains in respect to the character.

Fruiting competence is inherited as a quantitative character; in dikaryotic mycelia, poor fruiting potential is masked by high fruiting competence.

Five fruiting abnormalities, each genetically determined, have been analyzed and may be characterized as follows:

a. *Cauliflower,* a severely malformed, gill-less fruiting body; a quantitatively inherited character that is completely suppressed in matings with wild-type strains.

b. *Medusoid,* a profuse, irregular outgrowth of cylindrical projections (presumptive stipes?) that may open at their ends to produce small, normal fruits; a quantitatively inherited character that is only partially masked in matings with wild-type strains.

c. *Bug's ear,* a reduction of the fruiting body to a small (< 1 mm), ear-shaped fold, bearing hymenium on its lower, concave surface; a single-locus, dominant character.

d. *Coralloid,* a compact, convoluted, darkened fruiting body, sometimes expressed as a simple, microscopic, cup-shaped structure lined with hymenium (*micro-coralloid*); a single-locus, dominant character, always associated with a mycelial aberration and the production of indigo; either a pleotropic locus or three extremely closely linked loci; location known as to linkage group.

e. *Haploid (homokaryotic) fruiting,* variously expressed in different strains from only slightly stunted, gilled fruits to amorphous knots of compressed hyphae; sporulation sparse, spores mostly non-viable; a quantitatively inherited character distinct from dikaryotic fruiting competence.

The effects of light, nutrient, and other environmental factors have been again confirmed, but no restoration to normal fruiting in genetically inadequate stocks has been achieved through alterations of external factors.

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THE GENUS PHYSOPELLA (UREDINALES) REPLACES ANGIOPSORA [1]

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The genus *Physopella* was described by Arthur (**1**) in 1906. Although Arthur described telia, as well as uredia, he cited *Uredo vitis* Thuem. on *Vitis* sp. from Aiken, South Carolina as the type species. His description of the telia was drawn from a specimen (Sydow, Uredineen No. 1327) of *Phakopsora vitis* P. Syd. on *Vitis inconstans* (= *Parthenocissus thunbergii* Nakai) from Japan because no other telial specimen was available. As late as 1934, Arthur (**3**) wrote that "Telia . . . have only been collected in Japan. . . ." Since a telium-bearing type is required by the International Code of Botanical Nomenclature (**5**), the type of *Physopella* is *Phakopsora vitis* (now = *Phakopsora ampelopsidis*) and not *Uredo vitis*. In subsequent years *Physopella* was treated as a synonym of *Phakopsora* by P. and H. Sydow (**7**) and by Arthur (**2**). In 1934, Arthur (**3**) again used *Physopella vitis* for the grape rust.

Mains (**6**) in 1934 named the genus *Angiopsora* to accommodate several grass rust fungi whose telia are in subepidermal crusts and whose teliospores are catenulate in contrast to the irregular arrangement of the spores in the telia of *Phakopsora*. In 1949, Thirumalachar and Kern (**8**) discovered that there are rust fungi on dicotyledonous plants that have the same morphology as *Angiopsora* and they transferred several such species from *Phakopsora* to *Angiopsora*. They included in these transfers the grape rust as *Angiopsora ampelopsidis* (Diet. & P. Syd.) Thirum. & Kern, of which they considered *Phakopsora ampelopsidis* Diet. & Syd. (1898) and *Phakopsora vitis* P. Syd. (1899) to be synonyms. Their conclusion that the grape rust produces catenulate teliospores and hence corresponds, morphologically, to *Angiopsora* is correct. However, they overlooked, as did also Cummins and Stevenson (**4**), the fact that the transfer involved the type of the validly and previously published genus *Physopella*. Inasmuch as there seems to be no taxonomic basis for maintaining *Physopella* and *Angiopsora* as distinct genera, the latter must be reduced to synonymy.

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In order to correct the nomenclature of the species concerned the following new combinations are proposed:

Physopella africana (Cumm.) comb. nov.

Angiopsora africana Cumm. in Bull. Torrey Bot. Club **83**: 221. 1956.

Physopella ampelopsidis (Diet. & P. Syd.) comb. nov.

Phakopsora ampelopsidis Diet. & P. Syd. in Dietel in Hedwigia **37**: 217. 1898; *Phakopsora vitis* P. Syd. in Hedwigia Beibl. **38**: 141. 1899; *Physopella vitis* Arth. in Rés. Sci. Congr. Bot. Vienne, p. 338. 1906; *Angiopsora ampelopsidis* (Diet. & P. Syd.) Thirum. & Kern in Mycologia **41**: 288. 1949.

Physopella aurea (Cumm.) comb. nov.

Angiopsora aurea Cumm. in Bull. Torrey Bot. Club **83**: 221. 1956.

Physopella cameliae (Mayor) comb. nov.

Uredo cameliae Mayor in Mém. Soc. Neuch. Sci. **5**: 578. 1913 (telia present); *Angiopsora cameliae* (Mayor) Mains in Papers Mich. Acad. Sci. Arts, Letters **22**: 154. 1937.

Physopella clemensiae (Arth. & Cumm.) comb. nov.

Angiopsora clemensiae Arth. & Cumm. in Phil. Jour. Sci. **59**: 438. 1936; *Angiopsora cyrtococci* T. S. Ramak. & Sundaram in Indian Phytopath. **7**: 143. 1954.

Physopella compressa (Mains) comb. nov.

Puccinia compressa Arth. & Holw. in Arthur in Proc. Amer. Phil. Soc. **64**: 157. 1925, not Dietel 1907; *Angiopsora compressa* Mains in Mycologia **26**: 129. 1934.

Physopella digitariae (Cumm.) comb. nov.

Angiopsora digitariae Cumm. in Bull. Torrey Bot. Club **83**: 222. 1956.

Physopella hansfordii (Cumm.) comb. nov.

Phakopsora hansfordii Cumm. in Bull. Torrey Bot. Club **70**: 523. 1943; *Angiopsora hansfordii* Thirum. & Kern in Mycologia **41**: 286. 1949, not Cummins 1945; *Angiopsora hansfordiana* Joerst. in Ark. Bot. **3**: 574. 1956.

Physopella hiratsuksae (Syd.) comb. nov.

Angiopsora hiratsuksae Syd. in Ann. Mycol. **34**: 70. 1936.

Physopella lenticularis (Mains) comb. nov.

Angiopsora lenticularis Mains in Mycologia **26**: 127. 1934.

Physopella melaena (Syd.) comb. nov.

Phakopsora melaena Syd. in Ann. Mycol. **37**: 315. 1939; *Phakopsora randiae* Kern & Thurst. in Mycologia **36**: 508. 1944; *Angiopsora melaena* (Syd.) Thirum. & Kern in Mycologia **41**: 286. 1949.

Physopella melinidis nom. nov.

Angiopsora hansfordii Cumm. in Bull. Torrey Bot. Club **72**: 206. 1945.

Physopella meliosmae (Kus.) comb. nov.

Phakopsora meliosmae Kus. in Bot. Mag. Tokyo **18**: 148. 1904; *Angiopsora meliosmae* (Kus.) Thirum. & Kern in Mycologia **41**: 288. 1949.

Physopella pallescens (Arth.) comb. nov.

Puccinia pallescens Arth. in Bull. Torrey Bot. Club **46**: 111. 1919; *Angiopsora pallescens* (Arth.) Mains in Mycologia **26**: 128. 1934.

Physopella phakopsoroides (Arth. & Mains) comb. nov.

Puccinia phakopsoroides Arth. & Mains in Bull. Torrey Bot. Club **46**: 412. 1919; *Angiopsora phakopsoroides* (Arth. & Mains) Mains in Mycologia **26**: 128. 1934.

Physopella venezuelana (Syd.) comb. nov.

Phakopsora venezuelana Syd. in Ann. Mycol. **33**: 85. 1936; *Angiopsora venezuelana* (Syd.) Thirum. & Kern in Mycologia **41**: 287. 1949.

Physopella wiehei (Cumm.) comb. nov.

Angiopsora wiehei Cumm. in Bull. Torrey Bot. Club **79**: 213. 1952.

Physopella zeae (Mains) comb. nov.

Angiopsora zeae Mains in Mycologia **30**: 42. 1938.

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WOOD, TOMATO AND MALT EXTRACTS AND GROWTH OF SOME BASIDIOMYCETES

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(WITH 2 FIGURES)

In an earlier investigation reported from this laboratory (**10**) 190 of 310 isolations (281 species) of Basidiomycetes were found to grow poorly or not at all on a basal medium of asparagine, dextrose, mineral salts and purified agar. The poor growth of some of these fungi appeared to be caused by a lack of one or more vitamins of the B complex; others responded to the addition of vitamin-free casein hydrolysate to the basal medium; some required the addition of both vitamins and casein hydrolysate; a few grew well on malt extract but poorly on the basal medium supplemented with both vitamins and casein hydrolysate.

Yusef (**13**) investigated the nutritional requirements of 41 species or strains of *Polyporus* and *Fomes*, included in the survey mentioned above. All were found to be heterotrophic for thiamine, one for thiamine and pantothenic acid and one for thiamine and adenine. Substituting casein hydrolysate for asparagine and varying the amount of dextrose in the medium improved the growth of some species or strains. However, the growth of a number of species on malt extract was superior to that on any of the "synthetic" media tested. The identity of the "malt factor" was not determined.

The present report is concerned with the effect on the growth of a group of Basidiomycetes, of the addition of natural materials to a more complete medium [1] than one limited to mineral salts, dextrose and asparagine. The cultures used were obtained from various sources and

[1] The basal medium used in this investigation contained per liter 1.5 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 20.0 g dextrose, 2 g casein hydrolysate, 8.09 mg adenine sulfate, 2.22 mg cytosine, 4.11 mg guanine HCl, 2.72 mg hypoxanthine, 0.25 mg thymine, 2.24 mg uracil, 0.06 mg xanthine, 5.58 mg choline Cl, 0.62 mg orotic acid, 0.337 mg thiamine HCl, 0.376 mg riboflavin, 0.205 mg pyridoxine, 0.123 mg nicotinic acid, 0.476 mg calcium pantothenate, 0.137 mg *para*-aminobenzoic acid, 216.19 mg *m*-inositol, 1.0 mg folic acid, 0.01 mg biotin, 0.01 mg vitamin B_{12}, 0.005 mg B, 0.02 mg Cu, 0.1 mg Fe, 0.01 mg Ga, 0.01 mg Mn, 0.01 mg Mo and 0.09 mg Zn. The casein hydrolysate was "vitamin free," from General Biochemicals, Inc., and was neutralized with $CaCO_3$.

when individual fungi are mentioned specifically, the number under which they are maintained in the culture collection of The New York Botanical Garden is given.

EXPERIMENTAL

Each of 155 fungi, chosen mainly because they grew poorly or not at all on the agar medium containing mineral salts, asparagine and dextrose, was grown in duplicate in petri dishes on 2 percent malt agar and on an agar prepared with the more complete medium. Some of the isolations grew very slowly, even on malt agar. These included *Corticium investiens* (56445-R), *Fomes densus* (52046-S), *F. juniperinus* (CBS-6), *Hymenochaete rubiginosa* (58557), *Panus laevis* (71178), *Peniophora incarnata* (56449-S), *Phallus* sp. (S-1), *Polyporus circinatus* (72026a), *P. prunicola* (94048-S), *P. rutilans* (2287), *P. Schweinitzii* (71356-S), *P. squamosus* (39296), *Radulum orbiculare* (57402-S), *Sparassis crispa* (CBS-4), *Stereum sulcatum* (71329-R) and *Trametes americana* (18125-R). A number grew as well, or nearly as well, on the new basal agar as on malt agar. For the majority, however, growth on malt agar was superior.

From the latter, 82 isolations (73 species) were selected for further study. They were distributed according to genera as follows: 1 *Auricularia*, 1 *Claudopus*, 5 *Corticium*, 3 *Daedalea*, 1 *Favolus*, 11 *Fomes*, 1 *Ganoderma*, 4 *Hydnum*, 3 *Hymenochaete*, 1 *Hypholoma*, 3 *Lenzites*, 1 *Panus*, 3 *Peniophora*, 1 *Phlebia*, 3 *Pholiota*, 16 *Polyporus*, 10 *Poria*, 12 *Stereum* and 2 *Trametes*.

Each of these isolations was grown in duplicate or triplicate in 25 ml of liquid medium in 125 ml Erlenmeyer flasks at 20–25° C for a period of two weeks. The liquid media tested included the basal medium, 2 percent malt extract and the basal medium plus 2 ml per flask of wood extract or tomato extract. Flasks were inoculated with pieces of young mycelium about 1 mm in diameter, by the method of Yusef (**13**). Dry weights were determined by treating the mycelium with ethanol, filtering into Gooch crucibles, washing thoroughly with 70 percent alcohol and drying at 100° C.

The wood extract was prepared by autoclaving 500 g of dry beech wood (ground in a Wiley mill) with 10 l of distilled water. The filtrate was reduced on a hot plate to 1 l and filtered through celite. The final concentrate contained 10 mg dry matter per ml and 1 ml was equivalent to the extract of 500 mg of wood. Campbell's canned tomato juice was filtered through cheese cloth and celite. It contained 45 mg dry matter per ml. The reaction of the basal solution was pH 4.5±

and the addition of the wood or tomato extract had little effect on the hydrion concentration. The reaction of the malt extract was pH 4.6.

The effect of the natural supplements varied with the fungus and with the supplement. The growth of ten of the 82 fungi was no better in natural media than in the basal medium, but each of the other 72 grew substantially better in one or more of the supplemented media (for examples, see TABLE I).

TABLE I

DRY WEIGHT MG PER CULTURE AFTER TWO WEEKS GROWTH IN BASAL MEDIUM AND IN SAME MEDIUM PLUS TOMATO EXTRACT, WOOD EXTRACT, OR IN 2 PERCENT MALT EXTRACT. THE BASAL MEDIUM WAS SUPPLEMENTED WITH B-COMPLEX VITAMINS, CASEIN HYDROLYSATE AND PURINE AND PYRIMIDINE BASES

Fungus	Basal medium	Tomato extract	Wood extract	Malt extract
Corticium lividum (15084-R)	10	19	42	32
C. roseum (56489-S)	27	119	112	47
Daedalea confragosa (67939-S)	17	95	73	52
Favolus canadensis (52003)	9	9	24	8
Fomes fulvus (16870-S)	13	27	79	20
F. lobatus (71656-R)	24	160	133	76
F. ulmarius (CBS-3)	31	19	62	24
Hydnum chrysocomum (1605)	3	10	16	14
H. septentrionale (72067)	11	40	34	34
Peniophora coccineo-fulva (57418-S)	6	26	42	32
Polyporus fibrillosus (94071-R)	57	131	125	54
P. radiatus (71313-S)	5	24	24	12
P. Schweinitzii (86353)	3	28	15	4
Poria tenuis (67942)	3	18	27	18
Stereum rameale (57046)	13	34	69	23
S. rameale (86400)	41	83	78	38
S. subpileatum (50230-R)	14	51	20	22

Wood extract was the most generally beneficial; the growth of 71 of the isolations was significantly greater in the medium supplemented with wood extract than in the basal medium, and for 49 the dry weight in the wood extract medium was two or more times that in the basal medium. The wood extract in the amount used appeared to be somewhat inhibitory for two of the fungi, *Polyporus rutilans* (2287) and *Poria carbonica* (8444).

Tomato extract significantly increased the growth of 62 of the isolations, and for 36 the growth was doubled or more. The growth of *Fomes ulmarius* (CBS-3) and *Stereum rufum* was somewhat inhibited.

The malt extract was least effective. Only 41 of the isolations were significantly stimulated by the malt extract, and 11 showed evidences of some injurious effects. The growth of 26 was doubled or more than doubled as compared to that in the basal medium.

Twenty-seven showed a marked response to wood extract and much less to either tomato extract or malt extract in the amounts used; the growth of 5 was markedly stimulated by tomato extract but not by wood or malt extract.

In the above experiment one quantity only of each of the natural substances was tested. Since natural products may have both beneficial and detrimental effects on growth, the resultant depending in part on the amount used, we selected 13 fungi and grew each for two weeks in the more complete basal medium and in the same medium plus per flask (25 ml of medium), 0.05, 0.1, 0.2, 1.0 or 5.0 ml of wood extract; 0.25, 0.5, 1.0, 2.0 or 4.0 ml of tomato extract; or 2.5, 10.0, 50.0, 100.0 or 500.0 mg of malt extract. Generally speaking, the best growth was obtained with the largest amount of supplement (TABLE II), but the

TABLE II

DRY WEIGHT MG PER CULTURE AFTER TWO WEEKS GROWTH IN BASAL MEDIUM AND MAXIMUM DRY WEIGHT IN SAME MEDIUM PLUS VARIOUS AMOUNTS OF TOMATO EXTRACT, WOOD EXTRACT OR MALT EXTRACT. THE BASAL MEDIUM WAS SUPPLEMENTED WITH B-COMPLEX VITAMINS, CASEIN HYDROLYSATE AND PURINE AND PYRIMIDINE BASES

Fungus	Basal medium	Tomato extract	Wood extract	Malt extract
Fomes igniarius (94020)	5	14	21	8
F. officinalis (80518-S)	1	4	4	6
Hydnum coralloides (59062-S)	3	20	10	10
Hymenochaete rubiginosa (1650)	12	42	12	28
Peniophora coccineo-fulva (57418-S)	2	21	26	18
Polyporus radiatus (71313-S)	3	12	27	14
Poria ambigua (86357)	58	117	94	93
P. corticola (71280-R)	25	51	50	51
P. tenuis (67942)	2	30	24	15
Stereum fasciatum (52006-R)	3	36	34	30
S. subpileatum (50230-R)	11	92	25	21

results varied with the fungus and supplement. The growth of *Polyporus radiatus* (71313-S) was increased more by wood extract than by tomato extract or malt extract. On the other hand, the addition of tomato extract markedly improved the growth of *Stereum subpileatum* (50230-R) while wood and malt extract were relatively ineffective (FIG. 1). Tomato extract was more effective than wood or malt extract with *Hydnum coralloides* (59062-S), *Hymenochaete rubiginosa* (1650) and *Poria ambigua* (86357). *Stereum fasciatum* (52006-R) responded to all three supplements (FIG. 2).

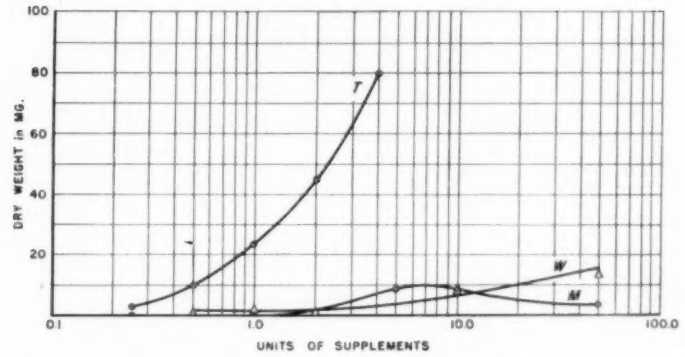

FIG. 1. Effect of addition of various amounts of tomato (T), wood (W) or malt (M) extract to a basal medium of mineral salts, dextrose, casein hydrolysate, purine and pyrimidine bases and B complex vitamins, on growth of *Stereum subpileatum*. Ordinate, net dry weight per culture; abscissa, units of extract added per flask (25 ml medium). Wood extract 1 unit = 0.1 ml = 1 mg dry matter. Tomato extract 1 unit = 1.0 ml = 45.1 mg dry matter. Malt extract 1 unit = 10.0 mg dry matter.

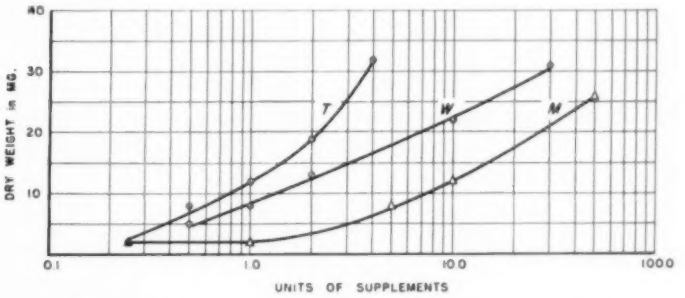

FIG. 2. Effect of addition of various amounts of tomato (T), wood (W) or malt (M) extract on growth of *Stereum fasciatum*. See legend for FIG. 1 for details.

DISCUSSION

It is not possible in a survey such as is reported here to go further than to indicate individual organisms which deserve further study and to formulate some of the problems which their responses present. The fungi we surveyed may be more or less definitely placed in the following groups.

1. Those which grew poorly on a mineral-salt-glucose-asparagine medium, but flourished on a mineral-salt, glucose medium with nitrogen supplied as casein hydrolysate and supplemented with various B-vitamins and purine and pyrimidine bases. For these, asparagine may have been a poor source of nitrogen and/or the supplements may have supplied deficiencies from which the organisms suffered. It would be a matter of routine to determine specifically the substances in our more complete medium responsible for the greater growth of these organisms.

2. Those which grew poorly on the more complete basal medium and on malt extract and on the other natural media tested. Their growth may have been limited by any one or more of a number of factors including temperature, the nature and quantity of mineral salts (**11**), carbon, nitrogen, or oxygen supply, osmotic relations, O-R potential (**4**), hydrion concentration, or essential unidentified metabolites not furnished in the media used. We cannot accept the statement sometimes made that such fungi are "naturally" slow-growing species. We assume that it is possible to define conditions which would allow more rapid growth of such organisms.

3. Those the growth of which was markedly improved by the addition to our more complete medium of wood, tomato or malt extract. The beneficial effect of wood extract on so many of the fungi we surveyed is noteworthy, especially because for some of them, including *Fomes ignarius, Polyporus radiatus, Poria tenuis, Peniophora coccineo-fulva* and *Stereum fasciatum*, the addition of 0.25 mg in dry weight of wood extract to 25 ml of medium caused a significant increase in growth.

We recognize that improvement in growth by natural materials may be due to several causes including the nature and quantity of mineral salts, carbon or nitrogen compounds furnished by the natural material, its effect on the hydrion concentration or OH potential, and the addition of unidentified growth substances. The action of wood extract in our experiments could not be accounted for by effect on the hydrion concentration, and in view of the composition of our basal medium to which the extract was added, would not seem to be due to the wood extract supplying mineral salts, carbon, or nitrogen. The possibility that the benefit from the natural substances is due to their supplying growth substances not included in our basal medium cannot be neglected.

The probability that heterotrophism for unidentified metabolites exists for a number of the higher fungi has been suggested by previous investigators. Fries called attention to the stimulating effect of malt extract and yeast extract on the growth (**1, 2, 3**) and spore germination of some wood destroying Basidiomycetes. Melin (**5, 6, 7, 8**) reported water

extracts of the litter under forest trees promoted the growth of mycorrhiza-forming fungi and found that pine and tomato roots had a strong stimulating effect on a considerable group of Basidiomycetes. He concluded that the stimulating material was not included among the known B-vitamins, purine and pyrimidine bases or casein hydrolysate. Yusef (**13**) called attention to an unidentified factor in malt. Robbins and Hervey (**12**) presented evidence for an unidentified water-soluble, thermostable growth factor for *Stereum murraii* present in natural materials, including wood. Nilsson (**9**) found 2.1 mg of fructose 1–6 phosphate per 100 ml to stimulate the growth of *Boletus variegatus* and *Collybia velutipes*.

We are inclined to believe that wood, tomato and malt extract contain unidentified growth substances which satisfy partial deficiencies from which some of the higher fungi suffer, although we have not in this investigation eliminated the possible effectiveness of other factors. The ultimate answer would, of course, be found in the isolation of such growth factors, if they exist. For this purpose organisms could be used which showed marked responses to tomato, wood or malt extract (TABLES I and II). However, it should be pointed out that for assay purposes the higher fungi included in this study have the disadvantage of requiring a week or more to evidence satisfactory growth responses.

SUMMARY

Of 82 Basidiomycetes selected because they grew better on malt extract agar than on an agar prepared with a basal medium of mineral salts, dextrose, B vitamins, purine and pyrimidine bases and casein hydrolysate, the growth of 72 was better when the liquid basal medium was supplemented with wood, tomato or malt extract. Wood extract was the most generally beneficial. For some of the fungi 0.25 mg in dry weight of wood extract in 25 ml of culture medium caused a significant increase in growth.

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NOTE ON LENZITES CINNAMOMEA FR.[1]

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(WITH 2 FIGURES)

While studying *Glocophyllum sepiarium* (Wulf. ex Fr.) Karst., I found, several times, specimens belonging to this species which, however, had been classified as *Lenzites cinnamomea* Fr.

Lloyd placed a note concerning the species with a specimen received by him from Malts of Latvia (Lloyd Coll. 55969) in which he said: "The latter [*Lenzites cinnamomea* Fr.] was named and determined by Fries from Sweden and Mexico, but no other specimens are found in the museums and now the color of these old specimens is all gone. No one ever found *Lenzites cinnamomea* Fr. but Fries. Fries gave an illustration of it in his Icones which illustrates the plant as bright zinc orange although the plant itself is much darker—russet of Ridgway. Mr. Malts sends this collection as *Lenzites saepiaria* (Wulf. ex Fr.) Fr., which it is in fact, but this common species always has parts that are brighter color than these. *Lenzites cinnamomea* Fr. of tradition (Fries Icones) bright zinc orange, does not exist I believe excepting in pictures."

Fries described *L. cinnamomea* Fr. in Öfv. K. Vet. Akad. Forh. **8**: 51. 1851, from material found on *Quercus* sp., in Smolandia. He suggested it as a rare species, very similar to *Lenzites betulina* (L. ex Fr.) Fr., in shape and size, but differing in internal color. Further examination of specimens in mycological herbaria reveals the name *L. cinnamomea* Fr. applied to specimens of *Glocophyllum trabeum* (Pers. ex Fr.) Murr., *G. abietinum* (Bull. ex Fr.) Karst. and *G. Berkeleyi* (Sacc.) Murr. Even the two collections in Bresadola's herbarium in the National Fungus Collections, named as such by him, are, respectively, *G. sepiarium* (Wulf. ex Fr.) Karst. (locality unknown) and *G. abietinum* (Bull. ex Fr.) Karst. (from Neapolim [?Neapolis], Italy).

For these reasons, until the opportunity occurred to examine authentic material from Fries's herbarium, we followed Karsten (1882)

[1] This work was done in the National Fungus Collections, Plant Industry Station, Beltsville, Maryland, with the assistance of a grant-in-aid from the National Council of Research of Brazil.

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and Fayod (1889) in their placement of the species in *Gloeophyllum*. Dr. Seth Lundell kindly sent to us at the N. F. C. a specimen of *L. cinnamomea* Fr. from the Fries herbarium, with the following remark: "The label is not in the writing of Elias Fries himself but of his son, Thore Magnus Fries."

This specimen of *L. cinnamomea* Fr. fits exactly the figure given by Fries in his Icones Hymen. **2**: *tab. 177, f. 2.* 1882. The specimen shows the following characteristics:

1—Macroscopic characters: Pileus sessile, coriaceous, rigid, 2.5 × 5 × 0.6 cm, brownish, zonate, hirsute-tomentose; context pale cinnamo-

FIG. 1. Upper surface and lamellate surface of *Lenzites cinnamomea* Fr. from Fries herbarium (Type).

meous, 0.2–0.3 cm thick; hymenial surface pale colored, lamellate, lamellae not equal, 11–13 lamellae per cm at the margin, 0.3 cm deep, 0.5 mm broad.

2—Microscopic characters: Hyphae—context presenting three kinds of hyphae: 1) generative hyphae, hyaline, clamped, branched, not thickened, 2.5–3.5 μ in diameter; 2) skeletal hyphae, hyaline (but appearing yellow in mass), non-septate, with hyaline thickened walls to subsolid and solid, sometimes with a colored content in the lumen, not branched, 3.5–9 μ in diam., more frequently 5–7.5 μ in diameter; 3) binding hyphae,

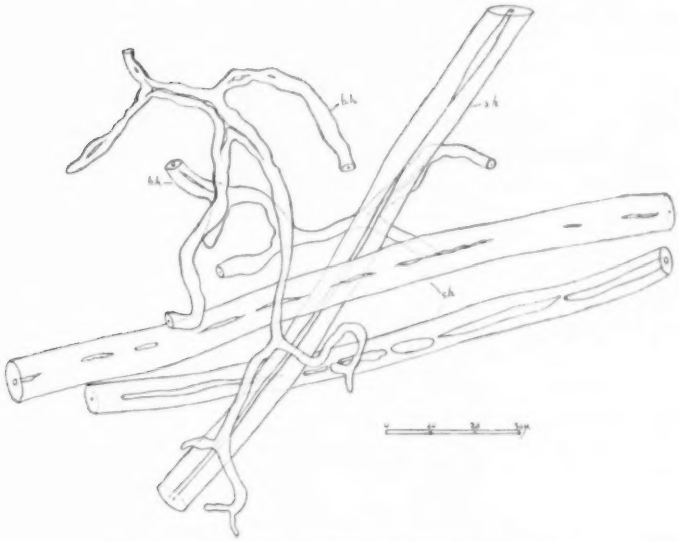

FIG. 2. b.h = binding hyphae; s.h = skeletal hyphae.

hyaline, strongly branched, coralloid, thickened to solid, non-septate, 1.5–4 μ in diameter. The first hyphal type is much less abundant than the binding and skeletal hyphae. Cystidia—fusiform, hyaline, with thickened walls, 19–25 × 4.5–5.5 μ. Spores—very few seen, hyaline, cylindric, 4.5–5.5 × 2 μ.

The hyphal analysis shows definitely that this species does not belong to *Glocophyllum*, contrary to Karsten's opinion (*G. cinnamo-*

TABLE I

		L. cinnamomea	*L. betulina*	*G. sepiarium*
Number of lamellae per cm		11–13	11–14	(14–) 15–26
Skeletal hyphae	Color	Hyaline	Hyaline	Yellowish brown
	Diameter	3.5–9μ	4–9μ	3–5.5μ
Binding hyphae	Branching	Strongly branched	Strongly branched	Poorly branched
	Diameter	1.5–4μ	2–3.5μ	2–3.5μ
Spores		4.5–5.5×2μ	4.5–6×1.5–2.5μ	7–10(–11)×3–4μ

meum (Fr.) Karst., Bidr. Känned. Finl. Nat. Folk **37**: 80. 1882), for the following reasons:

A—The walls of the skeletal hyphae of *Gloeophyllum* species are yellowish-brown to brown, never hyaline as in *Lenzites cinnamomea* Fr. In this species, the color of the context is not given by the walls of skeletal hyphae, but by their content, as occurs in some species with white context and especially in *L. betulina* (L. ex Fr.) Fr., in which, at times, the context is pale colored but the walls of skeletal hyphae are always hyaline.

B—The binding-hyphae of *L. cinnamomea* Fr. are of a very distinct kind, strongly branched, and not the incipient type, moderately branched, found in *Gloeophyllum* species.

Actually, I believe *L. cinnamomea* Fr. is really just a darkish form of *L. betulina* (L. ex Fr.) Fr., as can be seen in TABLE I.

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FUNGI ISOLATED FROM OAK-WILT TREES AND THEIR EFFECTS ON CERATOCYSTIS FAGACEARUM [1, 2]

ALEX L. SHIGO

(WITH 2 FIGURES)

Since the discovery of oak wilt in West Virginia in 1951, isolations have been made from a large number of oak trees each year and many fungi in addition to *Ceratocystis fagacearum* (Bretz) Hunt have been found associated with these trees. This fact is also recognized by investigators in other states who have made isolations from killed oak trees (Morris and Fergus, **1952**; Young and Brandt, **1953**; Turk, **1955**).

The current method of processing oak-wilt trees to control disease spread in West Virginia is by deep-girdling to the heartwood and allowing the trees to remain standing (True and Gillespie, **1956**). Thus, an abundance of naturally infected trees was available for a study of the succession of fungi in trees girdled soon after the appearance of oak-wilt symptoms. Fifty trees infected with oak wilt were left to stand ungirdled for a time as control for observations and experiments reported here.

The fungi growing in deep-girdled oak-wilt trees sometimes differed from those found in non-girdled trees. This was of especial interest since the development of *C. fagacearum* appeared to be partially checked following girdling.

The principal question considered in this investigation involved the possibility that the girdling of oak-wilt trees resulted in conditions that favored the growth of certain fungi antagonistic to the growth of *C. fagacearum*.

This paper reports the results of isolations, cultural studies, and artificial inoculations designed to give more information on the nature

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[2] Condensed from a Thesis submitted to the faculty of the Graduate School of West Virginia University in partial fulfillment of the requirements for the Degree of Master of Science.

of the fungi associated with trees killed by oak wilt and to suggest ways in which some of them may influence the development of *C. fagacearum* in girdled oak-wilt trees.

MATERIALS AND METHODS

To determine the identity of the fungi commonly associated with trees killed by oak wilt, and to determine whether these fungi had any effect on the development of *C. fagacearum* in the diseased trees, a study was undertaken along three lines: (1) A taxonomic study of the natural succession of fungi in oak-wilt trees was made by means of isolations from various parts of trees at intervals following the appearance of symptoms of oak wilt. (2) A physiological study was made in the laboratory of the fungi commonly isolated from oak-wilt trees. These fungi were cultured along and in association with *C. fagacearum* to determine the possible stimulatory or inhibitory effects involved in the association. (3) An ecological study was made to determine whether certain treatments applied to artificially inoculated oak-wilt trees would affect the growth and sporulation of *C. fagacearum*.

The first part of the program, and a portion of the third, included the collection of samples from various parts of trees killed by *C. fagacearum*. The majority of the samples brought into the laboratory for culturing were tangential slabs ten inches long and seven inches wide taken from the trunks. Portions of branches and roots were also collected. Samples were collected from 400 oak-wilt trees (300 girdled and 100 non-girdled), 30 deep-girdled healthy trees, 25 wounded healthy trees, and dead branches from ten healthy trees. Cultures were also obtained of fungi associated with a total of 175 mats of the oak-wilt fungus from 25 trees. Collections extended over a period of 18 months from June 1956 to December 1957 and included samples from all major oak-wilt areas in West Virginia.

Isolations of the fungi from the tree were made under aseptic conditions in the laboratory as outlined by Barnett (**1953**). A malt-yeast medium composed of 5 grams of malt extract, 1 gram of yeast extract and 20 grams of agar per liter of distilled water was used. The pH of the medium was adjusted to approximately 6.0 before autoclaving at 15 pounds steam pressure for 15 minutes. Approximately 8 small chips of wood from the debarked and surface-sterilized samples were placed on Petri dishes containing the above medium.

The fungi on the oak-wilt mats were identified, if possible, and then the surface-sterilized wood beneath the mats was cut into small chips

and placed in Petri dishes of the above medium. All instruments and samples were dipped in 95% alcohol and flamed. The cultures were then incubated at 25° C for 10 days before they were brought into the laboratory for examination. It was necessary to re-examine some cultures after a longer period of incubation in order to identify them.

The materials and methods used in the physiological study of the fungi and in the inoculation studies are presented in the sections reporting results of these experiments.

EXPERIMENTAL RESULTS

FUNGI ISOLATED FROM NATURALLY INFECTED OAK-WILT TREES IN 1956

Representatives of 65 genera of fungi were identified in the six-month period from June until December 1956 from 558 samples collected from 186 oak-wilt trees located at scattered points throughout the state of West Virginia. A list of the fungi frequently isolated is given in TABLE I. Most of the samples were collected from trees girdled by the recommended control method currently used in West Virginia (Gillespie *et al.*, **1957**). Collections were made at various periods after girdling. Samples were also collected from trees not having oak wilt (TABLE I, cols. 1 and 2).

Ceratocystis fagacearum was isolated more frequently than any other fungus from branches of oak-wilt trees sampled from June through September of both girdled and non-girdled trees. Trees sampled soon after symptoms were observed yielded the oak-wilt fungus in culture more frequently than any other fungus. Only 15 percent of 117 girdled trees sampled in November yielded *C. fagacearum* in culture. The oak-wilt fungus was isolated from the wood beneath only two of 58 mats cultured. Perithecia were observed on only 19 of the 165 mats examined.

Hypoxylon punctulatum (Berk. & Rav.) Cke. was isolated frequently from girdled oak-wilt trees sampled late in the year. Fifty-six percent of the 117 girdled trees sampled in November yielded this fungus. Stromata of the fungus were observed frequently in the field on oak-wilt trees girdled the previous year. Oak-wilt fungus mats were not observed on deep-girdled trees producing stromata of *H. punctulatum*.

Most of the isolates recorded in the data as *Dothiorella* sp. were later identified as *D. quercina* (Cke. and Ell.) Sacc., but some believed to be the same species failed to sporulate. This fungus was isolated frequently from samples collected in November from girdled oak-wilt trees. *Dothiorella* sp., *Graphium rigidum* (Pers.) Sacc. and *Hypo-*

TABLE I

NUMBER OF TREES YIELDING THE FUNGI MOST FREQUENTLY ISOLATED, FROM JUNE TO DECEMBER 1956

Fungus	JUNE THROUGH SEPTEMBER 1956						NOVEMBER AND DECEMBER 1956	
	Healthy trees		Oak-wilt trees				Oak-wilt trees	
			Branches		Trunks			
	Wounds on trunks (1)	Dead branches (2)	Non-girdled (3)	Girdled (4)	Non-girdled (5)	Girdled (6)	Trunks girdled from June to Aug. 1956 (7)	Wood beneath mats on girdled trees (8)
Ceratocystis fagacearum	0	0	6	3	14	15	17	2
Graphium rigidum	14	0	3	2	12	14	92	20
Dothiorella sp.	1	4	4	0	6	17	88	13
Hypoxylon punctulatum	1	0	0	0	2	9	66	7
Gliocladium roseum	1	0	0	1	8	11	47	17
Trichoderma lignorum	8	0	3	1	11	13	45	15
Penicillium sp.	3	0	1	1	9	16	30	13
Ceratocystis pluriannulata	8	0	2	0	7	10	28	13
Hypoxylon atropunctatum	0	5	6	2	11	11	14	0
Fusarium sp.	10	0	2	1	5	7	23	0
Cephalosporium sp.	14	2	0	1	5	13	37	7
Trichothecium roseum	0	0	0	0	0	0	1	10
Total trees	19	5	6	4	14	18	117	20
Total samples	36	10	19	9	41	51	273	58
No. Petri dishes examined	169	55	300	74	402	445	560	332

xylon punctulatum were often isolated from the same samples. *C. fagacearum* and *Dothiorella* sp. were often isolated from the same sample and neither seemed to have any antagonistic effect on the other in culture. *Dothiorella* sp. and *C. fagacearum* were the only fungi isolated from one small red oak that was producing oak-wilt fungus mats.

Graphium rigidum was isolated frequently from the wood beneath wounds on healthy trees and the wood beneath oak-wilt fungus mats. This fungus was isolated more frequently than any other from deep-girdled trees sampled in November.

Gliocladium roseum Bain. was observed growing on many oak-wilt fungus mats and was isolated from the wood beneath 49 of the 58 mats cultured. The antagonistic nature of *G. roseum* on other fungi was observed during the examination of cultures in Petri dishes.

Hypoxylon atropunctatum (Schw.) Cke. was isolated with great frequency from branches on healthy trees killed as a result of ice breakage and branches from non-girdled oak-wilt trees. It was isolated more frequently from trees sampled early in the season. *H. atropunctatum* and *C. fagacearum* were often isolated from the same samples and in such instances the latter was soon overgrown by the former species.

Trichoderma lignorum (Tode) Harz. was isolated frequently from the wood beneath wounds on healthy trees and from the wood beneath oak-wilt fungus mats.

Ceratocystis pluriannulata (Hedgc.) C. Moreau was isolated with moderate frequency from the wood beneath wounds made in healthy trees, girdled oak-wilt trees, and the wood beneath oak-wilt fungus mats.

Trichothecium roseum (Pers.) Link was isolated frequently from the wood beneath mats, but only once from the wood of trees not producing mats.

The following is a list of miscellaneous fungi of some interest that were isolated only a few times: *Calcarisporium parasiticum, Hyalopycnis blepharistoma, Petriella* sp., *Gelasinospora adjuncta, G. tetrasperma, Hypoxylon marginatum, Stysanus medius, Ceratocystis fimbriata, Curvularia lunata, Arthrobotrys oligospora, Nigrospora sphaerica, Beauveria bassiana, Sporocybe curziana, Stachylidium* sp., *Acrostalagmus* sp., *Oedocephalum* sp., and *Monochactia* sp.

EFFECTS OF SELECTED FUNGI ON C. FAGACEARUM IN CULTURE

It was observed during the examination of cultures isolated from oak-wilt trees that *C. fagacearum* could not compete successfully under these conditions with some of the other fungi frequently isolated from oak-wilt trees soon after wilt symptoms were observed.

Observations were made of *C. fagacearum* growing in combination with other fungi on several different media in Petri dishes and in some cases in liquid media in 250 ml Erlenmeyer flasks. The different media used were: three different concentrations in grams per liter of malt extract and yeast extract (20–2, 10–2, and 5–1); glucose-phenylalanine; and maltose-Casamino-Acids. (For composition of last two media, see Barnett, **1953**.)

An extensive study revealed that *Hypoxylon punctulatum, Dothiorella quercina, Graphium rigidum,* and *Ceratocystis pluriannulata* had little or no effect on the growth of *C. fagacearum* in culture. However, *Gliocladium roseum, Hypoxylon atropunctatum, Trichothecium roseum, Penicillium* sp. and *Trichoderma lignorum* overgrew *C. fagacearum* and either checked its growth or killed the mycelium.

On a maltose-Casamino-Acids medium essentially free of pyridoxine, *Graphium rigidum* produced synnemata only when it grew on or near a culture of *C. fagacearum* or *D. quercina.* These results suggest a nutritional association between these fungi.

Trichoderma lignorum produced more spores when it overgrew the oak-wilt fungus than it did in any other area of equal size on the medium. It is concluded that the mycelium of *C. fagacearum* contains some products that stimulate spore production by *T. lignorum. Trichothecium roseum* prevented the growth of *C. fagacearum* by producing an antibiotic that diffused through the medium (Turk, **1955**). *Hypoxylon atropunctatum* rapidly grew over the oak-wilt fungus and inhibited its further growth without evidence of a diffusable antibiotic. *Gliocladium roseum* was antagonistic on contact with *C. fagacearum* and all other fungi paired with it.

To determine the extent of the antagonism of *G. roseum* it was grown in combination with other species on the agar media used above. After a few days the mycelium and conidiophores of *G. roseum* were visible growing over all of the associated fungi, even though some of them grew more rapidly than *G. roseum* and soon covered the agar surface. Species readily attacked by *G. roseum* included *C. fagacearum, D. quercina, H. atropunctatum, T. lignorum,* and *T. roseum.* The principal effects of the attack were the collapse of the surface mycelium and the destruction of the dark pigment in the mycelium of some species. *G. roseum* was equally effective in overgrowing and destroying established cultures of the above species.

Of the fungi commonly isolated from the oak-wilt trees, *H. punctulatum* is of particular interest because of its possible effect on *C. fagacearum* in the dead trees. *H. punctulatum* was isolated from trunks

of 56 percent of the girdled oak-wilt trees sampled in November (TABLE I, col. 7). It caused a rapid rot of the sap wood. The effects of this association in the wood of dead trees are not known. However, on agar media *H. punctulatum* has no adverse effect on the growth of *C. fagacearum*. Only the conidial stage, belonging to the genus *Basidiobotrys*, has been produced in culture. The evidence that the perfect stage of this fungus is *Hypoxylon punctulatum* was presented by Barnett (**1957**).

RESULTS OF INOCULATION STUDIES—1957

Results obtained from previous field studies and work in the laboratory suggested that *Gliocladium roseum* is inhibitory to *C. fagacearum* and other fungi. To determine the effect of *G. roseum* on *C. fagacearum* in the tree, *G. roseum* was used to inoculate 62 of 204 red, black, and scarlet oak trees infected with oak wilt. These trees ranged from 3 to 15 inches d.b.h. and were previously inoculated during the last week of June with *C. fagacearum* in the following manner: The bark was removed from a small area of the trunk by means of a drawknife, taking care not to injure the xylem. The attachment surfaces of an inoculation cup made from aluminum sheeting were coated with caulking compound and placed tightly against the smooth-shaven area (FIG. 1). Approximately 25 ml of a dilute spore suspension was placed in the cup and the tree was wounded below the surface of the liquid by driving a chisel into the outer sapwood. Every tree inoculated in this manner developed oak-wilt symptoms in approximately three weeks. After oak-wilt symptoms were observed, *G. roseum* inoculum was placed in a sorbose-KNO_3 selective medium in a watertight plastic pouch surrounding the girdled portion of the trunk of 62 trees (Baer, **1958**) (FIG. 2). In laboratory tests the sorbose-nitrate medium permitted growth of *G. roseum* but was unfavorable for the growth of *C. fagacearum*. Fifty-two oak-wilt trees served as controls for the *G. roseum* inoculation group. The sorbose-KNO_3 medium alone was added in the plastic pouches. Forty-four oak-wilt trees were deep-girdled and received no further treatment. Forty-six oak-wilt trees received no treatment, and thirty oak trees not infected with *C. fagacearum* were deep-girdled. The percentage of defoliation at the time of treatment ranged from 20% to 95%, and the majority of the trees were over 50% defoliated when treated.

Two months after treatment the first samples were taken from the wood a few feet above the treated areas on the trunks of standing trees. *Gliocladium roseum* was reisolated from 38 of 62 sampled trees inocu-

lated with it. This fungus was isolated less frequently from the trees receiving other treatments. Regardless of the treatment, *C. fagacearum* was not isolated from any deep-girdled trees. *Hypoxylon punctulatum*

FIG. 1. Inoculation cup made from aluminum sheeting. Caulking compound coats the attachment surfaces, assuring a water-tight seal, and also holds the cup firmly on the smoothly shaven bark. Approximately 25 ml of a dilute spore suspension was placed in the cup and the tree was wounded below the surface of the liquid by driving a chisel into the outer sapwood. FIG. 2. Encircling plastic pouch on a deep-girdled oak-wilt tree containing *Gliocladium roseum* inoculum in a selective nutrient medium. Caulking compound is placed on the smoothly shaven bark below and above the girdle. Large rubber bands around the plastic pouch assure a watertight seal.

was isolated from almost every deep-girdled tree that did not have a plastic pouch over the girdle. This species was causing a rapid sapwood decay. *Dothiorella* sp. was isolated frequently from all treated trees. *Graphium rigidum* and *Trichoderma lignorum* were isolated from only a few trees.

TABLE II

FREQUENCY OF IMPORTANT FUNGI ISOLATED FROM ARTIFICIALLY INOCULATED OAK-WILT TREES AND HEALTHY TREES, THREE TO FOUR MONTHS AFTER TREATMENTS; NOVEMBER AND DECEMBER 1957

	Trees inoculated with *Gliocladium roseum* in a sorbose-KNO_3 medium			Trees serving as Controls and lacking *G. roseum* inoculum			Trees deep-girdled and receiving no other treatment			Healthy trees deep-girdled		
Portion of tree sampled yielding fungus 1. base 2. upper trunk 3. branches	1	2	3	1	2	3	1	2	3	1	2	3
Fungus												
Hypoxylon punctulatum	7	4	0	11	5	0	31	24	3	19	11	0
Dothiorella sp.	36	30	22	30	26	24	10	16	28	5	6	6
Penicillium sp.	11	16	30	7	17	27	4	4	17	7	14	20
Hypoxylon mediterraneum	10	10	14	3	9	20	3	4	19	3	4	14
Schizophyllum commune	2	5	20	2	4	15	1	4	15	0	3	15
Graphium rigidum	4	2	1	1	0	0	5	0	0	6	3	0
Gliocladium roseum	14	8	4	2	4	3	1	1	2	5	4	1
Ceratocystis fagacearum	10	14	12	9	14	14	0	0	5	0	0	0
Total no. of trees	52			59			39			28		

TABLE II lists the frequency with which the important fungi were isolated three to four months after treatment of the trees. All trees not having green and normally moist tissues above the deep girdle in November and December 1957 were then cut. Samples were collected from the lower and upper portions of the trunk and from branches. Fifteen sub-samples were cut from the four or more samples collected from each tree.

Gliocladium roseum was not isolated as frequently in the November-December sampling as it was in October. The samples were cut a greater distance from the point of inoculation than in the first survey. The reduction in the frequency of isolation of *G. roseum* indicates that the fungus did not spread far from the point of inoculation. *C. fagacearum* was isolated more frequently in November and December than in October and was recovered from green branches of many trees even though the trunks were dry.

Hypoxylon punctulatum was isolated more frequently from deep-girdled trees receiving no other treatment than from deep-girdled trees having a plastic pouch covering the girdle. This species was isolated more frequently from the lower part of the trunk than from any other part of the tree and was seldom isolated from small branches. These results suggest that the fungus entered the tree through the deep-girdle wounds and spread upward through the sapwood. *Dothiorella* sp. was isolated frequently from all trees except healthy deep-girdled trees. *Hypoxylon mediterraneum, Penicillium* sp. and *Schizophyllum commune* were isolated frequently from the branches of all trees. *Graphium rigidum, C. pluriannulata,* and *Trichoderma lignorum* were isolated from only a few trees.

Because of the scarcity of oak-wilt fungus mats, and the many trees still too green in November 1957 to produce mats, it is impossible to determine whether *G. roseum* affects the production of mats.

Although the results of these inoculation experiments did not answer the primary question, additional knowledge of the fungi isolated from oak-wilt trees has been obtained, and the results justify further study of the role of *Hypoxylon punctulatum* in deep-girdled oak-wilt trees.

DISCUSSION

It has been suggested by Jewell (**1955**) that a mutualistic symbiotic relationship has long existed between the nitidulid beetles and *Graphium rigidum* and *Ceratocystis pluriannulata,* and that the oak-wilt fungus is a recent addition to this long-standing association. Results obtained in this study support this theory. It was observed that the population and

frequency of isolation of fungi from deep-girdled naturally infected oak-wilt trees in 1956 differed from those isolated from deep-girdled, artificially inoculated oak-wilt trees in 1957. The most striking difference was the absence of *G. rigidum* and *C. pluriannulata* from many of the deep-girdled inoculated oak-wilt trees, while *G. rigidum* was isolated more frequently than any other fungus from deep-girdled, naturally infected oak-wilt trees. Under similar conditions with artificially inoculated trees in 1957, only 12 of 150 trees yielded *G. rigidum* in culture.

It should be pointed out that artificial inoculation of a tree with *C. fagacearum* is not the same as the natural vector inoculation. When the trees are artificially inoculated, other fungi commonly associated with the natural vector are not simultaneously introduced. This fact suggests a possible reason why results obtained from inoculation studies are not always comparable to natural conditions.

The high frequency of isolation of *Hypoxylon punctulatum* from both deep-girdled naturally inoculated and artificially inoculated trees indicates that deep-girdling creates conditions favorable for the entrance and establishment of the fungus. The low frequency of isolation of *C. fagacearum* from these trees indicates also that the conditions created by deep-girdling are not favorable for the growth and sporulation of the oak-wilt fungus (Gillespie *et al.*, **1957**).

Mixed-culture studies of *H. punctulatum* and *C. fagacearum* in the laboratory revealed that the two fungi grew well together on the agar media used. If the possibility can be excluded that *H. punctulatum* is directly antagonistic to the oak-wilt fungus in the trees and the fact considered that it produces a rapid sapwood decay, then this suggests that the decay caused by *H. punctulatum* and not the presence of the fungus itself is responsible for the inability of the oak-wilt fungus to grow and sporulate in deep-girdled trees. These data also suggest the possibility that there is strong competition in the sapwood for food. They justify a further study of *H. punctulatum*.

The failure of *Gliocladium roseum* to spread in the tree under conditions used in these experiments may explain why it was ineffective in checking the oak-wilt fungus in the tree.

Several dieback fungi were isolated from oak-wilt trees. *Dothiorella quercina* was isolated more frequently than any other dieback fungus. *D. quercina* and *C. fagacearum* were isolated frequently from the same sample and they grew well together in culture. In culture, *D. quercina* held back the growth of some fungi which are antagonistic to *C. fagacearum*. These results suggest that *D. quercina* may favor the growth, survival, and sporulation of *C. fagacearum* in nature. The fungi com-

mon on, or near oak-wilt fungus mats (*G. roseum, G. rigidum, C. pluriannulata, T. lignorum,* and *T. roseum*), all have spores well adapted for insect transmission and are probably introduced onto the mats by insects.

The diversity of the genera of fungi isolated from oak-wilt trees indicates a very complex association of fungi following infection of a tree by *C. fagacearum.*

SUMMARY

From June 1956 until December 1957 more than 400 oak-wilt trees in West Virginia were sampled. Approximately 5000 Petri-dish cultures from 1158 samples were examined in the laboratory. Most of the samples were collected from deep-girdled oak-wilt trees. The fungi most frequently associated with trees killed by *C. fagacearum* in West Virginia were identified. Representatives of over 50 genera were isolated from oak-wilt trees.

Some fungi isolated from oak-wilt trees were antagonistic to *C. fagacearum* in mixed cultures, while others were not.

Hypoxylon punctulatum was often isolated from deep-girdled oak-wilt trees, where it causes a rapid sapwood decay. Oak-wilt fungus mats were not observed on deep-girdled trees producing stromata of this fungus.

G. roseum destroyed all fungi that were paired with it in culture. It destroyed established Petri-dish cultures of *C. fagacearum. G. roseum* failed to inhibit the growth of *C. fagacearum* in trees, probably because it failed to spread within the trees.

Fewer genera of fungi were isolated from artificially inoculated oak-wilt trees than from naturally inoculated trees. *Graphium rigidum* in particular was isolated frequently from naturally inoculated oak-wilt trees but only a few times from artificially inoculated trees.

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AN INITIAL STUDY OF PATHWAYS OF CARBON METABOLISM IN HANSENULA ANOMALA (HANSEN) SYDOW[1, 2]

NORMAN D. DAVIS

The physiology of the yeast cell has been extensively studied by research workers in many areas of science. Most of these investigations have been with the classical *Saccharomyces cerevisiae* Hansen, probably because of its importance to the brewing and baking industries. Kuehner (**1951**) pointed out that the physiology of the wild yeasts has scarcely been studied, and cited the synthesis of ethyl acetate by *Hansenula anomala* (Hansen) Sydow as one example of the potential commercial importance of yeasts other than *Saccharomyces cerevisiae*.

Hansen (**1894**) reported that *Hansenula* species on fermenting media produce a fruity odor, and Klöcker (**1903**) suggested that the ester, which is responsible for the odor, might be ethyl acetate. Gray (**1949**) reviewed the literature of ester synthesis by *Hansenula* species and observed that, although the ester has been repeatedly referred to as ethyl acetate, no one had previously identified it by chemical means. Gray (**1949**), upon chemical analysis of the fermentation medium of *H. anomala*, identified ethyl acetate, ethyl alcohol, acetaldehyde, and acetic acid. He further investigated some of the conditions leading to ester synthesis, and established that the ester accumulated only under optimal conditions. He reported also that the ester, once formed, may be metabolized, disappearing from the medium at about the same rate as it was formed. Davies *et al.* (**1951**) confirmed Gray's report of the metabolic products formed by this organism, and reported that small amounts of glycerol were formed. Peel (**1951**) investigated ethyl-acetate formation by washed suspensions of *H. anomala* cells, using ethanol and acetate as carbon sources in the substrates. He reported that the ester may be formed with ethanol, or with ethanol and acetate as substrates, but not when acetate alone is the substrate. He concluded from equilibrium data that the ester is not formed by a Cannizzaro reaction of acetalde-

[1] Based on a portion of a dissertation submitted to the Graduate School of the Ohio State University in partial fulfillment of the Ph.D. degree.

[2] Paper No. 622 from the Department of Botany and Plant Pathology, the Ohio State University.

hyde, as suggested by Wolf and Wolf (**1947**). Peel also investigated the effect of *p*H, age of cultures, and ethanol concentration on ester synthesis. Hughes (**1952**) detected two acids in the fermentation medium of *H. anomala*. One he identified as acetic acid, while he reported the other as an unidentified non-volatile acid, confirming the report of Davies *et al.* (**1950**). Hughes reported it to be saturated, to contain only one acidic group, and to decompose without melting at 212°–217° C. Davies *et al.* reported that the acid is not pyruvate, lactate, or succinate.

Tabachnick (**1951**) presented an extensive literature review of ester synthesis by microorganisms, and reported his investigation of ethyl-acetate formation by *H. anomala* and *Pichia kluyveri* Bedford. He worked with growing cultures, washed-cell suspensions, and cell-free extracts, investigating numerous aspects of ester synthesis. He reported, as did Gray (**1949**), that excess aeration resulted in a decrease in ethyl-acetate formation. Tabachnick and Joslyn (**1953A**, **1953B**) presented evidence that the ester was later utilized in further metabolism. They used specific esterase inhibitors and demonstrated that ester formation was not the reverse of a simple hydrolysis involving esterase.

Brock (**1952**) investigated the effects of various inhibitors on respiration in *H. anomala*. He pointed out that certain inhibitors of glycolysis and the tricarboxylic acid cycle did not appear to inhibit respiration in this organism, and suggested the possibility that a pathway of carbohydrate metabolism similar to a Warburg-Lipmann-Dickens scheme (Lamanna and Mallette, **1953**) may be operative. It is this viewpoint that stimulated the present investigator to initiate this research. Brock (**1952**, **1956**) also investigated lipid synthesis in *H. anomala*, and reported on various factors that lead to the synthesis of large amounts of lipid in this organism. Och (**1956**) studied the growth of *H. anomala* with various amino acids as the source of both carbon and nitrogen and reported on similarities and differences of the known varieties and species of *Hansenula*.

The extent of the reported differences between *H. anomala* and *S. cerevisiae* makes the former organism worthy of additional investigation. This work was initiated for the purpose of investigating some of the pathways of carbon metabolism in *H. anomala*, with a view toward better understanding the mechanism of ethyl-acetate synthesis in this organism.

MATERIALS AND METHODS

H. anomala, strain No. 20 of the Ohio State University culture collection, was used throughout this work. The culture was maintained

on slants of medium containing: glucose, 2 g; agar, 2 g; Difco yeast extract, 0.7 g; KH_2PO_4, 0.5 g; in 100 ml of distilled water. Cells used for the respiratory studies were obtained by transferring a loopful of cells from the agar slants into 1000 ml Erhlenmeyer flasks containing 200 ml of the following medium: glucose, 5 g; Difco yeast extract, 0.7 g; KH_2PO_4, 0.5 g; in 100 ml of distilled water. The cultures in liquid medium were incubated for 48 hr at 26°–28° C. After incubation the cells were harvested by centrifuging for 10 min at 2500 rpm. In experiments with unstarved and unwashed cells, the freshly harvested cells were used immediately; however, in experiments with starved-cell suspensions, the harvested cells were first aerated for two hours in 0.02M KH_2PO_4, thus reducing endogenous respiration to a low value, and then either used immediately or stored in the refrigerator for not more than 24 hours. The respiratory studies were conducted at 27° on a shaking machine at a rate of 110 cycles per minute. Standard manometric techniques were used, the CO_2 values being obtained by the "direct" method of Warburg (Umbreit *et al.*, **1949**). All values for gas exchange have been calculated in μl of gas exchange per hour per mg dry weight of cells.

RESULTS AND DISCUSSION

TABLE I lists the results of experiments in which intermediates of the tricarboxylic-acid cycle were used as substrates. Some of the compounds were not oxidized under the experimental conditions used, although it is known that the organism can utilize these compounds for growth (Och and Davis, unpublished data).

TABLE I

OXYGEN UPTAKE BY WASHED, STARVED CELLS OF H. ANOMALA ON VARIOUS SUBSTRATES AT PH OF 6.0. NUMBERS REPRESENT μL OF GAS PER MG DRY WEIGHT OF CELLS PER HOUR

Substrate	QO_2
Endogenous	8.0
0.02M Glucose	28.8
0.02M Pyruvate	16.1
0.02M Oxalacetate	19.2
0.02M Citrate	16.1
0.02M Cis-aconitate	8.8
0.02M Isocitrate	17.5
0.02M Oxalosuccinate	19.3
0.02M α-Ketoglutarate	18.4
0.02M Succinate	9.2
0.02M Fumarate	8.6
0.02M Malate	10.2

TABLE II lists the results of experiments in which the 4-carbon tricarboxylic acids were used as substrates with and without added magnesium ion.

TABLE II

OXIDATION OF SUCCINATE, MALATE AND FUMARATE BY WASHED CELLS OF H. ANOMALA WITH AND WITHOUT ADDED MAGNESIUM ION (0.0025 PPM AS $MgCl_2$)

Substrate	QO_2	Per cent increase
Endogenous	8.0	—
Endogenous and magnesium	11.2	40.0
Succinate 0.02M	8.7	—
Succinate 0.02M and magnesium	14.2	63.2
Fumarate 0.02M	8.6	—
Fumarate 0.02M and magnesium	10.9	26.7
Malate 0.02M	10.2	—
Malate 0.02M and magnesium	12.5	22.5

Goucher and Kochalty (**1954**) suggested that phosphate buffers inhibit succinate oxidation when used for repeated washing of cells, and that the failure of the cells to oxidize succinate is not merely the result of the leaching of magnesium from the cells. It was noted in this work that iron or manganese ions did not reverse the inhibition to any extent as did magnesium ion. Unwashed and unstarved cells oxidize succinate, malate, and fumarate rapidly and to the same extent as they oxidize glucose. It was not determined whether the reduced ability to oxidize the acids is due solely to the phosphate-buffer wash.

It appears that several intermediate compounds of the tricarboxylic-acid cycle are not oxidized by cells that are washed and starved in phosphate buffers. It is possible that this phenomenon can be used to ad-

TABLE III

OXIDATION OF SUBSTRATES BY WASHED AND STARVED CELL SUSPENSIONS OF H. ANOMALA. NUMBERS REPRESENT GAS EXCHANGE PER MG DRY WEIGHT OF CELLS PER HOUR AT PH 6.0

Substrate	QO_2	QCO_2	R.Q.
Endogenous	8.0	6.4	0.81
Glucose 0.02M	28.8	44.1	1.50
Glucose-6-phosphate 0.02M	23.9	20.8	0.87
Fructose-6-phosphate 0.02M	15.1	13.6	0.90
Fructose-1,6-diphosphate 0.02M	15.2	13.1	0.86
Glyceraldehyde-3-phosphate 0.02M	13.8	12.1	0.88
Glycerol 0.02M	22.2	15.4	0.69
Pyruvate 0.02M	16.4	20.2	1.23
6-phosphogluconate 0.02M	21.8	24.0	0.99
Ribose-5-phosphate 0.02M	31.2	23.1	0.74
Ethanol 0.02M	23.7	9.6	0.40

vantage in studying the formation of ethyl acetate from glycolytic and monophosphate intermediates without introducing the complicating factors of the tricarboxylic-acid cycle. TABLE III lists the results of experiments in which intermediates of glycolysis and of the hexose-monophosphate pathway are used as substrates.

Pathways of carbohydrate dissimilation based upon respiratory quotients obtained experimentally are at best highly theoretical. However, it was observed that only one feasible pathway could be inferred from the data in TABLE III that would account for the R.Q. values obtained experimentally when ribose phosphate and phosphogluconate were used as substrates. This pathway involves the splitting of pentose into 3-carbon and 2-carbon fragments, the 2-carbon fragments being oxidized, possibly to acetic acid or to glycolaldehyde, and the 3-carbon fragments (phosphoglyceraldehyde?) being converted to ethanol and then to ethyl acetate by a mechanism similar to the one first suggested by Wolf and Wolf (**1947**). The respiratory quotients calculated for such a pathway are compared in TABLE IV with those obtained experimentally with several substrates.

TABLE IV

COMPARISON OF CALCULATED AND EXPERIMENTAL RESPIRATORY QUOTIENTS FOR THE SYNTHESIS OF ETHYL ACETATE BY H. ANOMALA FROM DIFFERENT SUBSTRATES AT PH OF 6.0

Substrate	Calculated R.Q.	Experimental R.Q.
Phosphogluconate 0.02M	0.96	0.99
Ribose-5-phosphate 0.02M	0.70	0.74
Phosphoglyceraldehyde 0.02M	0.93	0.88
Ethanol 0.02M	0.40	0.40

The R.Q. values obtained experimentally for fructose phosphate, fructose diphosphate, and glucose phosphate were intermediate to values calculated for these compounds when (1) they are oxidized solely through the hexose-monophosphate pathway or (2) they are oxidized solely through the classical pathway of glycolysis. The presence of the enzyme aldolase could not be demonstrated, possibly an indication that the hexose-monophosphate "shunt" is a major pathway of hexose dissimilation in this organism. The calculated R.Q. value for the oxidation of glucose through the monophosphate pathway is 0.80, and is identical to the experimental value obtained for endogenous respiration. The values obtained when glucose was the substrate varied from 1.0 at low concentrations to 1.5 or higher when high concentrations were used. It appears that other pathways of glucose dissimilation are operative when high concentrations of this substrate are present. The rapid oxi-

dation of the pentose-phosphate intermediates is believed to be significant, and to indicate that the monophosphate pathway to ethyl acetate is of major importance in the organism.

Several respiratory "poisons" were observed to inhibit the oxidation of the various substrates previously tested. The oxidation of glucose, glycerol and phosphogluconate was reduced 50 percent by a complex of 0.03M sodium fluoride and 0.04M phosphate buffer. The oxidation of pyruvate was not inhibited by the complex. Bisulphite inhibited the oxidation of all of the compounds used including ethanol. It appears that some of the ethanol is oxidized to acetic acid, which then reacts with ethanol to form ethyl acetate. Wolf and Wolf (**1947**) first suggested this possibility. Tabachnick and Joslyn (**1953**) presented evidence that the ester may be formed from ethanol alone, from ethanol and acetic acid, but not from acetic acid alone. Pathways of ethyl-acetate formation proposed by previous workers generally involve the dissimilation of hexose through glycolysis and ethanol formation. The results obtained here, using inhibitors of glycolysis and of ethanol synthesis, appear to confirm this pathway to ethyl acetate, except that the hexose-monophosphate pathway is thought to play an important role in the initial dissimilaion of hexose.

SUMMARY

It was determined that *Hansenula anomala* (Hansen) Sydow is able to oxidize and utilize for growth intermediates of glycolysis, of the monophosphate pathway, and of the tricarboxylic acid cycle. Phosphogluconate and ribose-5-phosphate are oxidized at a rate equal to or greater than the hexose phosphates of glycolysis, indicating that the hexose-monophosphate pathway of glucose dissimilation may be important in this organism.

The 4-carbon intermediates of the tricarboxylic acid cycle were oxidized only slightly or not at all if the cells were first washed and starved in a phosphate buffer. This treatment did not prevent the oxidation of glycolytic or monophosphate intermediates. Thus it appears possible to study the mechanism of ethyl-acetate synthesis in the absence of an active Kreb's cycle. The addition of magnesium ion to the reaction mixture significantly increased the rate of oxidation of succinate, and affected to a lesser degree the rate of endogenous respiration, malate oxidation, and fumarate oxidation.

Respiratory quotients obtained using monophosphate and glycolytic intermediates as substrates are interpreted and a pathway of ethyl-acetate synthesis is inferred. This pathway is similar to that suggested by previous workers, except that the monophosphate pathway is thought to be

a major pathway in the initial dissimilation of glucose, and that the pentose is split into 2-carbon and 3-carbon fragments with the 3-carbon fragments being converted to ethyl acetate via ethanol.

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SOME COMMENTS ON THE ASCOCARPS OF THE PYRENOMYCETES

LENNART HOLM

INTRODUCTION

Ever since von Höhnel (**1907**) established the family Pseudosphaeriaceae on the basis of his comprehensive studies on the anatomy of the fruit-bodies, such anatomy has been accorded paramount importance for the taxonomy of the Pyrenomycetes. It may be superfluous to recall here the lively discussion following this pioneer work; it was characterized by a good deal of terminological dimness, not least on the part of von Höhnel himself. Extremely important for later research has been Miller's dissertation (**1928**), a consequent and admirable attempt to bring clearness to the definitions. Miller's views, further generalized by Nannfeldt (**1932**), have on the whole been accepted by modern mycologists.

Luttrell's great review (**1951**) no doubt is the most important recent work devoted to these problems. He gives a critically commented upon and highly useful survey of the extensive literature on the ascocarps of the Pyrenomycetes, especially the works dealing with developmental studies. On the basis of this review in addition to his own investigations, Luttrell distinguishes 8 different types of ascocarps, which he finds characteristic of different groups of Pyrenomycetes. He takes a partly rather critical attitude to Miller's definitions but nevertheless accepts them as essentially correct.

In connection with several years' investigations on the taxonomy of the Pyrenomycetes, especially the Pleosporaceae, I have been confronted with these problems. In my recent thesis (**1957**) some terminological questions were briefly touched upon, but a more exhaustive treatment was beyond the scope of that work. The following pages may be looked upon as a supplement. They were originally conceived independently of Luttrell's work but have of course been stimulated by it, though they give expression to a partly deviating view. It may be convenient to begin with a short recapitulation of the current definitions of the two fundamental concepts, *stroma* and *perithecium*.

STROMA

The term "stroma" was introduced by Persoon; a detailed account of its meaning and use in older mycology was given by Orton (**1924**). In modern literature the term is generally used in conformity with the views set forth in great detail by Wehmeyer (**1926**) and Miller (**1928**), laying stress upon the vegetative character of the formation in question. Thus Wehmeyer defines the stroma as "an aggregation of vegetative mycelium not resulting from a sexual stimulus" (**1926**, p. 579) and Miller uses almost the same wording: "fungous bodies which are formed of coalesced hyphae, which do not arise as a result of a sexual stimulus" (**1928**, p. 191). It should be observed that these definitions do not exclude the possibility that even ascocarps (and pycnidia) may be stromatic to a larger or smaller extent.

PERITHECIUM

The term "perithecium" is now generally used in conformity with Miller's definitions (**1928, 1949**). According to these the perithecium originates entirely from the "archicarp" [1]: "a true perithecium; that is, walls arising from an archicarp, soon forming a globose hollow ball with a hymenium of asci and apically free paraphyses developing from a wall layer, and an ostiole formed by extension of the wall tissue and lined with periphyses" (**1949**, p. 108). "The ascocarp wall is a special structure developed from the basal cells of the archicarp and is not part of the stroma" (**1949**, p. 103).

So far Miller. For the moment I will make only one comment on his definition—that it does not seem quite clear if the presumed origin of the perithecium is the fundamental principle of this definition; in other words, if "perithecium" is a term for the archicarp and the hyphae growing out from it, and if in consequence the other characters of the perithecium are of secondary importance. This may be the explanation why Miller, as a matter of fact, does not present any convincing arguments for this alleged origin of the perithecium: this would be superfluous if it follows from the definition.

[1] The term "archicarp" was introduced by de Bary (**1884**) as a general term for egg cells and female gametangia in the plant kingdom on the whole. The archicarp of the Ascomycetes is denominated "ascogon" by de Bary. Therefore he can write (p. 214): "An der Bildung des Hüllapparats . . . bleibt das Archikarp unbeteiligt," which now sounds somewhat peculiar. Miller means by "archicarp" the whole ascogone-supporting, often spirally coiled little hypha (ascogonium + sterile supporting cells), and it is in this wide sense that the term is now mostly used.

As to the other characteristics of the perithecium, Miller writes (**1928**, p. 194): "To the writer the perithecial wall seems to afford a character which is definitely correlated with characters of the perithecial centrum. . . . When there is a true wall there is no pseudoparenchym in the centrum, there are true paraphyses and periphyses, concave hymenial layer, and the ostiolum is schizogenous in type." We will now confront these definitions with some more recent investigations on the origin and structure of the ascocarps of the Pyrenomycetes.

THE ONTOGENY OF THE PERITHECIUM

As mentioned above, Miller strongly emphasizes that the perithecium is derived wholly and completely from the archicarp. However, a number of investigations are now forthcoming, decidedly indicating that fruit-bodies generally regarded as perithecia in reality are of twofold origin: partly from the basal cells of the archicarp but partly also from its mother hypha or from other vegetative hyphae.

This was early pointed out by Dangeard in his classical work on the ontogeny of the perithecia (**1907**), and among more recent investigations affirming this view we may mention some studies on the Sordariaceae, such as those of Dodge (**1935**), Dengler (**1937**), Moreau (**1950**, **1951**) and Zickler (**1952**). Of special interest, however, is a study by Luttrell (**1944**) on perithecium formation in the nectriaceous fungus *Sphaerostilbe aurantiicola*. This species is stromatic just as are most of the Xylariaceae which are the starting-point for Miller's discussion. And moreover this study is performed by an investigator who has devoted himself especially to morphological questions; most studies of this kind are carried out by cytologists.

In *Sphaerostilbe* an archicarp is formed in the stroma, and this archicarp is eventually surrounded by a perithecial wall. Luttrell states about its origin: "Branches of the surrounding vegetative hyphae invest the ascogonium to form the perithecial wall" (**1944**, p. 617).

The investigations mentioned thus do not confirm Miller's definition, but on the contrary disagree with it. And in fact Miller probably regards these "perithecia" as at least in part stromatic, as is evident from the following:

It is a common condition in the Pyrenomycetes that the outer layers of the ascocarp wall are formed by rounded, rather large, often thick-walled cells, while the inner layers are composed of narrow, thin-walled, often hyaline cells. This is beautifully demonstrated, *e.g.*, by a figure in Shear & Dodge (**1927**, *pl. 4*) of some *Neurospora*-species, to which Miller refers: "The illustrations of perithecia of *Neurospora* by Shear

and Dodge . . . show typical perithecia, in which there is a definite wall, and also a thin pseudoparenchymatous layer on the outside, just as in species of *Rosellinia*. This pseudoparenchyma is stroma" (**1928**, p. 202). Miller indeed thinks that *all* representatives of the Sphaeriales and Hypocreales have a stroma: "In fact the perithecium in these two orders is, contrary to previous conceptions, apparently always formed within a more or less well-developed stroma" (**1928**, p. 193). He expresses the same opinion in 1949: "perithecia one to many in a stroma" (**1949**, p. 123).

One could then perhaps vindicate the opinion that the ascocarps in, *e.g.*, *Sordaria* and *Gnomonia* have a dual character, depending on their presumed twofold origin. The outer peridial layers—according to Miller stromatic—should then derive from vegetative hyphae, while the inner layers—that is the "true" perithecial walls—should derive from the archicarp. However, this statement is quite arbitrary; there is to my knowledge no proof of that being the case. Moreover the term "perithecium" then would lose its practical significance, and could no more be used for the ascocarps of the Sphaeriales and Hypocreales, if these were largely stromatic. However, Miller is, as far as I can find, practically the only one who takes the extreme view that the ascocarps of *Sordaria et alia* are partly stromatic.

But I will emphasize that even if with Miller one regards the outer peridial layers of *Sordaria* as not belonging to the perithecium *sensu stricto* one is *not* therefore entitled to consider them as stromatic, as does Miller. For all the fruit-body is no doubt the "result of a sexual stimulus."

In this connection it may perhaps be justified to recall some obsercations by Dengler (**1937**) concerning perithecium formation in *Sordaria Brefeldii*. This is stated often to be initiated by an anastomosis between ordinary hyphae: "Zwei gleich aussehende Mycelhyphen bilden eine Anastomose. Auf einem späteren Stadium sieht man alsdann, wie von den Nachbarhyphen an diese Anastomosen dünne Hyphen heranwachsen und die Anastomose allmählich völlig mit einer dichten Hülle umgeben. Diese Hülle bildet sich alsdann . . . in ein Perithezium um" (p. 437). If this is correct, the perithecia would be formed without an archicarp's being formed at all. The perithecium then would be completely stromatic!

However, it seems to me to be of doubtful value to uphold this sharp distinction between hyphae originating from the basal part of the archicarp and other vegetative hyphae, and I agree with Nannfeldt's words (**1932**, p. 19): "Im übrigen kann ich keinen tiefgehenden, prinzipiellen

Unterschied zwischen den vor der Anlage des Archicarps gebildeten Geweben und denjenigen Geweben anerkennen, die nach dessen Anlage aus vegetativen Zellen entstehen. Beide gehören ja der Einkernphase [homocaryotic phase] des Pilzes an."

OSTIOLE

The perithecium is said to be characterized by the presence of an "ostiolum"—*i.e.*, a pore formed schizogenously and lined with periphyses. There are, however, ascocarps lacking an ostiole which are generally regarded as perithecia—in part even by Miller himself. We will consider some examples of this.

The most striking one is offered by the group Erysiphales. Perithecium development of the powdery mildews has been the subject of a number of researches and is probably better known than in any other Ascomycetes. On the whole, throughout the group the archicarp (like the antheridium) is formed on free hyphae, and the ascocarp, at least in the main, is formed by hyphae issuing from the archicarp, or more exactly, from the ascogonial stalk cell. It is a moot question whether hyphae from the archicarp play such an important rôle in the formation of the ascocarps in any other Ascomycetes. The fruit-bodies consequently ought to be perithecia. This is also the case, according to Miller (**1928**, p. 211): "The Erysiphaceae have a true perithecial wall, but the lack of an ostiolum should be sufficient to place them in a distinct order." In his paper of 1949 Miller does not treat the Erysiphales more particularly but one gets the impression that he no longer regards their ascocarps as perithecia. Nannfeldt (**1932**, p. 31) counts them among the Ascoloculares, albeit this does not seem reconcilable with the fact that in this group "soviel bekannt ist, liegen jedoch die Ascogone in einem stromatischen Gewebe eingeschlossen" (p. 26). Luttrell regards the ascocarp of the Erysiphales as a perithecium (**1951**, p. 101).

An analogous example is afforded by the group Plectascales. Here, too, the ordinary condition seems to be that ascocarp formation is initiated by the forming of an ascogonium (and sometimes an antheridium) that is later surrounded by a dense hyphal ball. The origin of these hyphae is evidently somewhat different in different species; a very careful and detailed report of the conditions in *Aspergillus Fischeri* was given by Olive (**1944**): "Some of these hyphae are branches of the ascogonial coil itself; some arise from the same hypha which gave rise to the coil; while the majority probably grow in from neighboring hyphae" (p. 270). These lines could just as well have reference to a *Sordaria*. Are these fruit-bodies perithecia? They lack an ostiole as

well as paraphyses. It is difficult to get an idea of what Miller thinks about the nature of these ascocarps, but he says about "Eurotiales": "stroma lacking" (**1949**, p. 123). Luttrell calls them without reservation perithecia (**1951**, p. 90). Here I would point out that Luttrell apparently also means that the ascocarps of *Elaphomyces* are perithecia, an opinion which is, as far as I know, very uncommon. There is evidently a universal unreadiness to name those fruit-bodies "perithecia," presumably because of their size. Neither does one want to call them "stromata," and the consequence is that in the literature they pass as "ascocarps," "fructifications," "Fruchtkörper" et cetera.

A further case of non-ostiolate perithecium-like ascocarps was recently reported by Cain (**1956**). He describes a new genus *Tripterospora*, related to *Sordaria*, which Cain puts into a family of its own, *Tripterosporaceae*. This is distinguished from the *Sordariaceae* by the lack of an ostiolum and paraphyses: "Here, then, we have three species whose inostiolate ascocarps would place them, as mentioned above, in the present-day order Plectascales. But the 'cleistothecium' of *Tripterospora* develops from an ascohymenial type of ascogonium, coiled and twisted, and at approaching maturity encloses clavate asci with a thickened apical ring such as are characteristic of *Podospora* (*Sordaria*)" (p. 704). These fruit-bbdies are indisputably homologous with those of *Sordaria*, and they seem to show convincingly that the presence of an ostiole is of rather secondary importance. Ascocarps of essentially the same nature may have or lack an ostiole.

Finally, it seems highly doubtful that the ostiole is always schizogenous in origin. Several figures in the literature indicate that it may be more or less lysigenous. Moreover, this point is perhaps of less interest. The essential thing is that the periphyses evidently arise *de novo*, in the pore.

PARAPHYSES

The above-mentioned cases of "atypical" perithecia without an ostiole are at the same time examples of perithecia without paraphyses. There are, however, also "typical" ostiolate perithecia which nevertheless lack paraphyses, or at least paraphyses in the common sense. Thus, according to Miller (**1949**), the perithecia of the *Hypocreales* lack paraphyses but have instead so-called pseudoparaphyses. (Munk, **1954**, calls them "apical paraphyses" and evidently regards them as true paraphyses.) The existence of pseudoparaphyses in a perithecium is really very remarkable and Miller also points out (**1949**, p. 117): "Here, then, are wall and ostiole characters of the Sphaeriales combined with centrum

characters of the Pseudosphaeriales." This opinion is shared by Luttrell (**1951**).

ASCOSTROMA

The ascostroma is generally sharply contrasted to the perithecium. The fruit-body is here principally stromatic and in its origin is thus "not resulting from a sexual stimulus." The sexual processes take place at a later stage, in the more or less fully developed stroma. A perithecial wall is lacking and "the characters that accompany the lack of a perithecial wall are the presence of pseudoparenchyma in the centrum, absence of paraphyses and periphyses, the convex to flat or concave form of the hymenial layer, and the lysigenous type of the opening" (Miller **1928**, p. 194). We will later examine the validity of these criteria but will first pay some attention to the formation of the ascostroma.

THE ONTOGENY OF THE ASCOSTROMA

Stromata may be essentially different as to nature and genesis: either—and perhaps usually—plectenchymatous or parenchymatous. That ascocarps as well as pycnidia may be formed through 3-dimensional cell division was long ago realized by de Bary, and he designated them as meristogenous in contrast to the symphogenous—*i.e.*, the normal—plechtenchymatous ones. The existence of meristogenous ascocarps has since been demonstrated by a number of investigators in several genera; with no pretensions to completeness may be mentioned among more recent studies *Glonium* (Luttrell **1953**), *Pleospora* (Wehmeyer **1954**, Moreau **1956**), *Pseudoplea* (Müller **1951**, Wehmeyer **1955**), and *Sporormia* (Delitsch **1926**, Arnold **1928**, Page **1939**, Moreau **1956**). Kempton's important researches on the formation of pycnidia should also be mentioned (**1919**).

The difference between meristogenous and symphyogenous stromata may seem profound but has not, at least in general, been accorded any greater taxonomic importance. However, Nannfeldt (**1932**, p. 28) has stressed the occurrence of true parenchyma (stromata and spores) in the Ascoloculares.

In the cases mentioned the archicarp is apparently formed inside the ascostroma, as is to be expected. There are, however, certain observations indicating that even fruit-bodies generally considered stromatic may "arise as a result of a sexual stimulus." Very interesting conditions are reported by Higgins in his studies on three species of *Mycosphaerella* (**1920**, **1929**, **1936**). In these an ascogonium with a tricho-

gyne is already formed at such an early stage that only by straining the term can one speak of a stroma: "At the time the carpogonia are formed the young perithecial primordium consists of a rather loose weft or knot of hyphae" (**1929**, p. 291). Similar conditions have later been described by Jenkins in two other species of *Mycosphaerella* (**1938, 1939**). Luttrell has observed this and points out the resemblance in ascocarp formation between *Neurospora* (*sitophila*) and *Mycosphaerella tulipiferae*, investigated by Higgins, but epitomizes: "Even in such closely similar forms as *N. tetrasperma* and *M. tulipiferae* there is a slight difference in origin of the tissues forming the perithecial wall and those forming the outer layers of the ascostroma, and Miller's (**1928**) distinction between the perithecial wall and stromal tissues may be accepted as valid. This distinction in such an extreme example as that of *N. tetrasperma* and *M. tulipiferae*, however, is based merely upon a slight difference in time of origin of the two tissues, and the significance of this difference might be questioned" (Luttrell **1951**, pp. 72–73). This difference will be infinitesimal when we examine two other species, often regarded as stromatic—viz., *Perisporium funiculatum* and *Cochliobolus sativus*.

A developmental study of *Perisporium funiculatum* was published by Beatus (**1938**). His interesting results seem to have been but little noticed. The ascocarp is initiated by the formation of an ascogonium (and an antheridium) on free hyphae. The gametangia are then surrounded by a hyphal ball, which according to Beatus has a twofold origin, in part from the ascogonial stalk cell, in part from other neighbouring hyphae: "Ausser den Zellen, die der Mutterhyphe des Oogoniums entspringen, sind aber auch noch benachbarte Hyphen am Aufbau des Fruchtkörpers beteiligt" (p. 319).

Cochliobolus sativus, an important pathogen on several cereal crops, was recently thoroughly investigated by Shoemaker (**1955**), who reported several interesting and partly surprising results. The species is heterothallic. Ascogonia with long trichogynes are formed freely on ordinary hyphae and give rise to so-called protothecia—*i.e.*, ascocarpial primordia. These develop further only if the ascogonium has been dicaryoticized, usually by spermatia, which are formed in pycnidia. It is not evident from Shoemaker's paper whether the protothecium is formed by ordinary vegetative hyphae or by hyphae from the archicarp, or possibly has a twofold origin. Apart from this it is striking to what a great degree this ascocarpial development is similar to *Neurospora*'s. Still there is no doubt that *Cochliobolus* is a pleosporaceous genus, and the ripe ascocarps are of the normal *Pleospora* type, with pseudoparaphyses, etc.

If we sum up the above, we find here fruit-bodies that seem to be stromatic in nature, but whose development no doubt is the "result of a sexual stimulus."

ASCOSTROMA WITH OSTIOLE?

According to Miller *et al.*, the stromatic fruit-bodies open through a lysigenous pore. There are, however, several observations indicating that there may be exceptions. Thus Higgins describes in his *Mycosphaerella* species a schizogenous pore with periphyses. In several Pleosporaceae there are periphyses—or at least hyphae looking like periphyses, and which reasonably should be called so—in the pore which certainly is mainly lysigenous (Holm **1957**). This is particularly clear in several species of *Nodulosphaeria*, the periphyses of which have developed into coarse, persistent bristles (cf. Holm **1952**, fig. 5).

CONCLUSION

As this brief survey will indicate, several statements in the literature are not reconcilable with the current conceptions of perithecium and ascostroma. Perhaps some of the papers referred to should be taken *cum grano salis*, and confirming and completing studies seem to be highly desirable; but in any case one should be justified in stating that:

1. Ascocarps regarded as perithecia may lack an ostiole or paraphyses or both, and may at least in part be formed by ordinary vegetative hyphae.

2. Pseudoparaphyses are found in perithecia (Nectriaceae) as well as in ascostromata (Pleosporaceae).

3. Ascocarps regarded as stromatic may have periphyses and may arise through "a sexual stimulus."

One may wonder, then, if it is possible to find any tenable differences between perithecia and ascostromata. This was recently discussed by Doguet (**1956**), who studied the ontogeny of the nectriaceous genus *Neocosmospora*. He summarizes (p. 369): "Il ressort de cette comparaison qu'une seule différence peut être retenue entre les histoires des ascocarpes des *Pleospora* et des *Neocosmospora:* le massif cellulaire dans lequel apparait l'ascogone est surtout parenchymateux chez les premiers, surtout plectenchymateux chez les seconds. Est-ce suffisant pour appeler l'un stroma et pseudothèce, l'autre périthèce? Nous pensons que la séparation fondamentale entre les deux types de développement est, dans le cas présent, beaucoup plus théorique que réelle."

Interesting in this connection is Björling's investigation of the ontogeny of *Erysiphe graminis* (**1946**). This evidently differs considerably from the normal *Erysiphe* scheme. No gametangia are observed, and according to Björling the ascocarps are stromatic. The formation of an apical palisade-tissue of down-growing hyphae, which strikingly remind one of pseudo-paraphyses is especially interesting. Björling does not employ this term, but he points out the similarity with the corresponding structures in *Spororma*. It is undeniably quite surprising to find such an ascocarpic development in a group like the Erysiphales, which in other respects is so homogeneous. One is tempted to question the importance of ontogeny for the understanding of the morphology and taxonomy. The earlier mentioned *Perisporium* and *Cochliobolus* invite the same heretical attitude. *P. funiculatum* is no doubt related to *Spororma*, but the ascocarp development seems very widely different. In *Perisporium* free gametangia are formed which later are surrounded by hyphae, in *Spororma* the fruit-bodies are true parenchymatic stromata. Quite the same contrast exists between *Cochliobolus* and *Pleospora*, which certainly are rather closely related.

Thus it would seem as if in related groups the ascocarps could be in certain respects very different. On the other hand we meet in certain respects an extensive ontogenetic similarity between the taxonomically widely different *Neurospora* and *Cochliobolus*.

The main impression of this study is, I think, unfortunately rather disappointing: the situation seems confusing. This is easy to demonstrate but more difficult to remedy. I will, however, finish these lines with a reflection. Miller's definitions are based on the conditions in the Xylariaceae. Here we have a very beautiful and pronounced duality between stroma and perithecium. But are all stromata necessarily homologous? Could perhaps the ascostroma of the Pleosporaceae have a closer affinity to a perithecium than to the stroma of the Xylariaceae? In any case this seems conceivable if we give up the claim that the perithecium should derive exclusively from the archicarp. A support for this idea is possibly offered by the genus *Valsaria*. According to Munk (**1953**, p. 129) this genus belongs to the Ascoloculares. The ascocarps then should be stromatic, but they are in turn united in a stroma. I have not myself studied *Valsaria*, but if Munk's statements are correct, we have here two different kinds of stromata!

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NOTES AND BRIEF ARTICLES

On the Relation of the Sexual and Nonsexual Phases of Gonapodya [1]

Early in 1954 Johns and Benjamin (**2**) described the true nature of the sexual mechanism in *Gonapodya*. It was shown, for both *G. prolifera* (Cornu) Fischer (**1**) and *G. polymorpha* Thaxter (**10**), that the sexual act involves the union of a small motile antherozoid with a relatively large nonmotile egg [2] and that, following the fusion of the gametes, the zygote, after a variable period of amoeboid movement, is actively motile for a more or less extended period of time during which it is propelled by the persistent male flagellum as in *Monoblepharella*. The zygote eventually comes to rest, the flagellum is absorbed, and the spore is surrounded by a smooth thickened wall. Zygote germination was not observed.

The female gametangium of *Gonapodya* typically produces several eggs, often as many as 18–20, which usually are discharged from the gametangium prior to fertilization. This is in marked contrast to *Monoblepharis* (**4**, **9**) and *Monoblepharella* (**5**, **6**, **7**, **8**) in which a single egg per gametangium is the rule, although in the latter genus as many as three or rarely more eggs may be delimited occasionally. In *Monoblepharis* and *Monoblepharella* the eggs appear always to be fertilized within the oogonium; in *Monoblepharis* the zygote is nonmotile, maturing in place, or is briefly motile, oozing from the oogonium and encysting at the orifice of the latter; in *Monoblepharella* the zygote

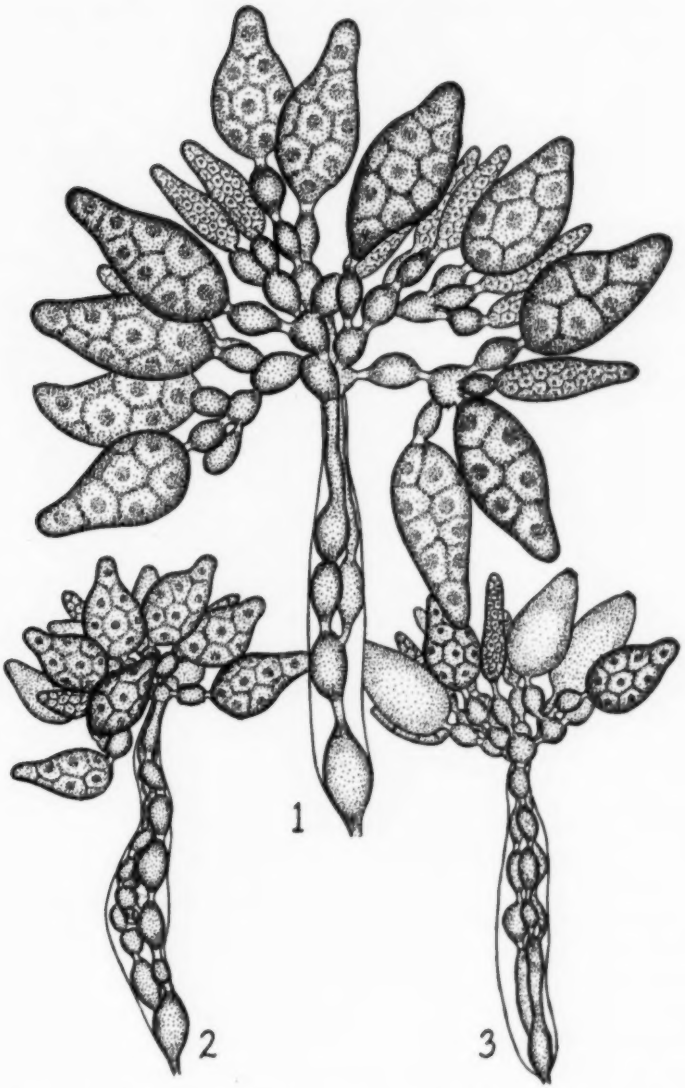
[1] The observations reported here were made during the tenure of the writer as a National Research Fellow in Botany, The Biological Laboratories, Harvard University, 1951–1952.

[2] In this connection it is interesting to note that Thaxter's description of *G. polymorpha* undoubtedly was based, at least in part, on sexual material, and his choice of the specific epithet, *polymorpha*, reflected the variation which he observed in the size of what now are known to have been ♂ and ♀ gametes (**10**, *Pl. 31, fig. 14*) rather than nonsexual zoospores. Because of the great discrepancy in spore size Thaxter (**10**, p. 479) at first suspected the presence of antherozoids, but, in his efforts to relate the genus to the Leptomitaceae, he described and illustrated a singular type of oospore (**10**, *Pl. 31, fig. 16*), later considered by Minden (**3**) as possibly belonging to *Pythiogeton*, which, although he could not demonstrate any organic connection, he thought might represent the sexual phase of *G. polymorpha*.

typically leaves the oogonium and, as in *Gonapodya*, enjoys a more or less protracted period of motility prior to encystment.

The account of sexual reproduction in *Gonapodya* presented by Johns and Benjamin (**2**) did not include information on the relationship between the sexual and asexual phases of either of the species studied. Investigations of *Monoblepharis* (**4, 9**) and *Monoblepharella* (**5, 6, 7, 8**) have shown that a given vegetative thallus normally produces both sexual and nonsexual reproductive structures. In these genera zoosporangia and gametangia often develop simultaneously, but the former may be produced in greater abundance at a somewhat lower temperature than that which is optimum for the development of the latter. Observations made during the course of the writer's study of the sexual phase of *Gonapodya prolifera* would seem to indicate that the nonsexual and sexual reproductive structures of this species, as in other members of the Monoblepharidales, are produced on the same vegetative thallus.

The author's observations on *G. prolifera* and *G. polymorpha* were carried out using living material which developed in the laboratory on oak twigs collected in April, 1952, in a shallow pond located in Middlesex Fells Reservation, near Winchester, Mass. The twigs, cut from a small oak tree that apparently had been felled into the pond during the previous growing season (an abundance of mature leaves on the tree would seem to bear out this hypothesis), were kept in a closed jar of pond water and stored in a refrigerator until needed. When these were transferred to distilled water in petri dishes and maintained at room temperature the characteristically pseudoseptate mycelia of both *G. prolifera* and *G. polymorpha* bearing an abundance of gametangia usually appeared in about three or four days. Both male and female gametangia borne singly or in fascicles of several elements were produced terminally by the extensive and usually highly branched thallus; in no instance were nonsexual zoosporangia formed by either species under these conditions of culture. Usually the mycelium appeared to arise directly from the substrate, but in *G. prolifera* several preparations, including those illustrated in FIGS. 1–3, showed quite conclusively that hyphal systems bearing gametangia had arisen by proliferation from the bases of empty, withered, siliquaeform structures of a shape characteristic of the zoosporangia of this species. The latter were interpreted as the remains of zoosporangia which had been formed by and had remained attached to a still living vegetative mycelium developed in the substratum during the previous growing season. No evidence was found to indicate that the sexual mycelia had arisen from resting bodies formed within the old zoosporangia.



FIGS. 1–3. *Gonapodya prolifera*. Three zoosporangia showing the internal origin, by basal proliferation, of vegetative mycelia bearing male and female gametangia (FIG. 1, × 420; FIGS. 2–3, × 265).

Pending a more thorough study of the life history of species of *Gonapodya* in pure culture, the writer feels that the observation cited above provides good circumstantial evidence that both sexual and non-sexual reproductive structures are, indeed, produced by the same vegetative thallus in this as in other genera of the Monoblepharidales.—R. K. BENJAMIN, Rancho Santa Ana Botanic Garden, Claremont, California.

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ANOMALOUS PHALLOIDS

It is of some interest to observe variations from the normal within any group of organisms, whether induced or occurring in nature. In Baton Rouge, on November 21, 1957, in a partially wooded section being exploited for home sites, a phalloid population was found in an area about 15 feet square. The fungi were growing mostly in and around a mound of earth covered by weeds, dead branches and building debris. Some were still in the egg stage and others had already emerged. In all, 72 specimens were collected, 61 of which were eggs. Of the 11 identifiable members, 7 were *Clathrus columnatus* Bosc and 4 *Mutinus Ravenelii* (Berk. & Curt.) E. Fisch. The 61 eggs were placed in moist

FIG. 1. Two normal collections of *Mutinus Ravenelii*. FIG. 2. Trifurcate specimen of *Mutinus Ravenelii*. FIG. 3. Three fruiting bodies of *Mutinus Ravenelii* showing deep furrows in the glebal region. All figures × ⅓.

chambers and within 24 hours, 16 had fully developed. Of this number, 4 were of special interest (FIGS. 2, 3). Subsequently, of the remaining total of 45, 32 normal specimens emerged, 18 of them *Clathrus columnatus* and 14 *Mutinus Ravenelii*. Thirteen did not develop, perhaps because of their small size at time of collection or because of previous injury. FIG. 2 shows a trifurcate anomaly of *Mutinus Ravenelii* which had the reddish coloration, spore size ($4–5 \times 2–2.5\ \mu$) and fetid odor usually associated with this species. Each of its finger-like extremities bore a spore mass, the distribution of which may be seen in the figure. The fruiting body was hollow and each branch had a pore at its extremity. The superficial resemblance of this specimen to a species of *Lysurus* or *Anthurus* (depending upon one's interpretation of these genera) is apparent. However, evidence indicating that the branched individual is an anomaly may be seen in FIG. 3 in which the 3 specimens, in spite of their obvious departure from normal morphology, are easily recognizable as *Mutinus Ravenelii*. The glebal region of each of these fungi has a deep furrow, which, had it penetrated the fruiting body and extended to the apex, would have produced a bifurcate individual. It seems likely, therefore, that the trifurcate specimen originally had 2 furrows, the further development of which later divided the apex into 3 parts. Although the fungi in FIG. 3 were somewhat smaller than the trifurcate and the normal ones (FIG. 1), typical coloration, spore size and odor were noted in all.—B. LOWY, Department of Botany, Bacteriology and Plant Pathology, Louisiana State University, Baton Rouge.

THE BULLER MEMORIAL LIBRARY [1]

On the afternoon of May 30th, 1958, a group of about fifty friends of the late Dr. A. H. R. Buller met at the Canada Department of Agriculture Research Laboratory in Winnipeg to attend the official opening of the Buller Library.

Dr. Buller was Professor of Botany at the University of Manitoba from 1904 to 1936. Although a man of simple tastes, he allowed himself one luxury: he collected a splendid personal library. It is mainly mycological, with valuable original editions of the works of Boudier, Tulasne, Brefeld and Zopf, but it also contains more general literature.

In 1923, following the epidemic of stem rust of wheat in Western Canada, Buller helped to establish the Dominion Rust Research Labora-

[1] A full account of the proceedings, prepared by Doctors Johnson and Sackston, will appear in the Commonwealth Phytopathological News.

Opening of the Buller Library, May 30, 1958. Some of Dr. Buller's Friends, Photographed after the Opening Ceremonies.—1. J. N. Welsh. 2. J. E. Machacek. 3. Mrs. Wright (Kirk Scott). 4. B. Peturson. 5. R. F. Peterson. 6. R. D. Bird. 7. W. L. Gordon. 8. F. J. Greaney. 9. Mrs. Keeping (E. Silver Dowding). 10. E. C. Stakman. 11. K. W. Neatby. 12. Ruth Macrae. 13. Marie McPhillips. 14. Margaret Dudley. 15. A. W. Alcock. 16. H. H. Saunderson. 17. T. C. Vanterpool. 18. Mrs. Yelland (Gladys Tremayne). 19. Airdrie Cameron. 20. W. E. Sackston. 21. D. L. Bailey. 22. J. H. Craigie. 23. Frank D. White. 24. W. A. F. Hagborg. 25. W. F. Hanna. 26. T. Johnson. 27. W. Popp. 28. ?. 29. E. Leith. 30. Frank Allen. 31. W. R. Allen. 32. W. J. Cherewick.

tory, and afterwards he became intimately associated with the research carried out there. The trustees of his estate therefore considered it appropriate to house his books in the Rust Research Laboratory. It was not until 1957, however, thirteen years after his death, that the Department of Agriculture Research Laboratory, newly erected nearby, furnished the suitable fireproof room to accommodate them.

With the growth of Buller's international reputation, he was awarded many honorary degrees, and he came to hold presidential and vice-presidential positions in many learned societies including one in the Mycological Society of America. Those who met in Winnipeg that day did not enlarge on Buller's professional honors. Rather they described small incidents illustrating his character as they remembered it. particularly his integrity, his enthusiasm, and his wit.

Dr. K. W. Neatby, Director of Science Service, Ottawa, was chairman of the meeting. He introduced the principal speaker, Dr. E. C. Stakman of the University of Minnesota, who described Buller's ever-youthful curiosity; his fresh, original approach in solving problems; and his total lack of authoritarianism in dealing with his students. Dr. Stakman hoped that the Library would inspire men to work in the same spirit.

Dr. T. Johnson, Chief of the Canada Department of Agriculture Research Laboratory in Winnipeg, read letters from Dr. Buller's friends living in other countries who were unable to be present at the meeting.

Dr. W. F. Hanna, Chief of the Botany and Plant Pathology Division of the Canadian Department of Agriculture, deposited Dr. Buller's ashes in a receptacle in the wall of the library. A bronze tablet inscribed: *A. H. Reginal Buller, 1874–1944,* closed the receptacle. A portrait of Dr. Buller hangs above it.—E. SILVER DOWDING (Mrs. E. S. Keeping), Department of Botany, University of Alberta, Edmonton, Alberta, Canada.

MANUSCRIPT

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